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Studies of Cyprinodont Fishes. XXI. *Glaridodon latidens*, from Northwestern Mexico, Redescribed and Referred to *Poeciliopsis*[1]

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(Plate I; Text-figure 1)

Introduction

Among the most troublesome jobs faced by the systematist is the proper allocation of old species based solely on insufficient and imperfect type material from an unknown or indefinite locality. One such species, *Glaridodon latidens* Garman, from "Chihuahua, Mexico," has posed an enigma since no specimens fitting the description have heretofore been brought to light from northern Mexico. The generic placement of this fish has always remained in doubt.

On the basis of a critical comparison of the four female type specimens with the extensive series of *Poeciliopsis* in the University of Michigan Museum of Zoology (UMMZ), we identify *Glaridodon latidens* with confidence as a valid species of the genus *Poeciliopsis*. It is assumed that the type locality of *P. latidens* is not in the Rio Grande drainage, as has commonly been supposed, but is probably in the headwaters of the Rio del Fuerte. This Pacific-drainage stream rises in Chihuahua, although the material we interpret as *latidens* is entirely from the adjacent states of Sonora and Sinaloa, through which this stream and others inhabited by the species also flow. It is one of the common poeciliids in the Pacific drainage of northwestern Mexico.

Acknowledgments

We are greatly indebted to the authorities of the Museum of Comparative Zoology (MCZ) of Harvard College, and particularly to Mrs. M. M. Dick, for a loan of the types of *Glaridodon latidens* and for catalogue data concerning these specimens. We also wish to thank the authorities of the Chicago Natural History Museum (CNHM), and especially Loren P. Woods, for the loan and exchange of comparative material. Boyd W. Walker, of the University of California at Los Angeles, kindly sent us series of *Poeciliopsis* from northwestern Mexico. The photographs were taken by Photographic Services, University of Michigan, and slightly retouched by William L. Brudon, artist of the University of Michigan Museum of Zoology. Funds for obtaining the photographs were provided through a research grant to the junior author from the Horace H. Rackham School of Graduate Studies.

Status of Glaridodon latidens

Types.—The existing type material (MCZ No. 1307) consists of four adult females, about 22

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 681, and from the Museum of Zoology, University of Michigan.

to 25 mm in standard length. The specimens are stained brown, somewhat shrivelled, and the fins are mostly broken. One individual has half of the lower jaw pulled off and another has both jaws pulled off, presumably by Garman when he examined the dentition (see Garman, 1895, Pl. V, Fig. 11). There is a small hole through the side of each specimen, and one has been dissected on the left side to expose the vertebrae.

Type Locality.—In response to a query regarding the type locality, Mrs. Dick wrote on December 5, 1952, as follows:

The specimens of *G. latidens* were catalogued by F. W. Putnam in April, 1861, as being from Chihuahua, Mexico, and the preceding entry, 1306, also was recorded from there, with a reference to an original number of 1 for 1307 and 1-3 for 1306. These must be original numbers in a small collection for they are not numbers used by Agassiz, the Smithsonian or any other source that I can run down. I can't find any previous record of any fishes from Chihuahua in Putnam's records or catalogues. Evidently these fish were specimens he found here since they are not on any of the lists of specimens received previous to 1861. There is no record of the collector or of the date of collection.

In his account of this species, listed as *Glaridichthys latidens,* Meek (1904, p. 134) wrote:

I do not know this species, and I am inclined to think there is some mistake in the locality given for it. I do not believe this genus is represented in northern Mexico.

The genus *Poeciliopsis* is almost wholly restricted in its distribution to the Pacific drainage, where it ranges from Arizona to Colombia, although it is replaced by related genera in Panama and the adjacent part of Costa Rica. On the Atlantic slope it is not known north of the basin of the Rio Chachalacas, in Veracruz, Mexico (Plan del Rio; material at the University of Michigan), although the area between Veracruz and Chihuahua has been well explored ichthyologically. It is therefore natural that the designated locality of Chihuahua for *latidens* has been open to doubt. However, study of the drainage relationships shows that a considerable area in eastern and southeastern Chihuahua drains into the Pacific, chiefly through Rio Yaqui and Rio del Fuerte, but in small part also through Rio Sinaloa and Rio Culiacan. Since Meek (1904, p. xxxviii) collected in the headwater portion of the Yaqui and reported only *Poeciliopsis occidentalis* for that region, and since extensive collections made by Ralph G. Miller and John T. Greenbank and party in the middle and lower parts of the same river contain no species comparable to *P. latidens,* it is concluded that the types of that species were not collected in the Rio Yaqui system. If correctly referred to Chihuahua, they probably came from the upper part of the Rio del Fuerte although no collections are at hand from this section of the river. Lower in the same stream, in both Sonora and Sinaloa, a species we identify as *Poeciliopsis latidens* is common. This species also occurs in the Sinaloa and Culiacan systems, but it seems improbable that the types were taken in either, for the areas they drain in Chihuahua are small, sparse in population, relatively inaccessible and very probably too high and mountainous for this type of fish.

Poeciliopsis latidens (Garman)

Glaridodon latidens.—Garman, 1895, p. 42, Pl. V, Fig. 11, teeth (original description; Chihuahua, Mexico).

Glaridichthys latidens.—Meek, 1904, p. 134 (account taken from Garman; type locality questioned). Regan, 1906-08, p. 99 (account taken from Garman). Regan, 1913, p. 1002 (species listed without description).

Poeciliopsis latidens.—Hubbs, 1926, pp. 66-67 (comment on type locality; referred to *Poeciliopsis* on basis of description only). Jordan, Evermann & Clark, 1930, p. 189 (listed only). De Buen, 1947, p. 279 (provisionally listed; range given, obviously by presumption, as "Cuenca del río Bravo, en río Conchos (Chihuahua)"). Alvarez, 1950, p. 88, footnote (status not clear; unidentifiable from original description).

A critical study of the four types of this species demonstrates clearly that it is referable to the Poeciliidae, even though the type series includes no males. Each of the four females shows characteristics that separate the Poeciliidae from the other cyprinodont families. The first three rays of the anal fin are unbranched, the third ray reaches nearly to the tip of the fin, and the fourth ray is widely branched. The neuromasts or pit organs are arranged along the axial scale row posteriorly and, on the caudal peduncle, along the second row above the midventral row. The two series of scales with pit organs are separated, as in most poeciliids, by one row of unpored scales. Anteriorly, some neuromasts occur on scales in the next row above the axial series and in irregular locations above the anal fin and thence forward to below and behind the pectoral fin. The second scale row above the axial series may also have a few neuromasts near the dorsal fin (this is not a family character).

Although fully reliable generic identifications in the Poeciliidae can be made only from males, the general resemblance of the types of *Glaridodon latidens* to species of *Poeciliopsis* led the senior author to refer *latidens* correctly to that

genus. This generic reference, however, would remain in doubt were it not for the remarkably close correspondence between the types of *latidens* and a *Poeciliopsis* that is common in northwestern Mexico (from near Alamos, Sonora, southward to Mazatlan, Sinaloa).[2] *P. latidens* is a banded or spotted species with the outer row of firm teeth arranged in a broad arc. Of the species heretofore referred to this genus, only *P. fasciata* (Meek), from the Pacific drainage of southern Mexico, fits this type, but the correspondence is not as close with *fasciata* as it is with the similar species in northwestern Mexico that we identify as *latidens*. The two forms are closely related but appear to be specifically distinct.

Description of the Four Types.—Fin rays: dorsal 7 and anal 9 in all (since Garman counted the two elements of the last ray separately, he reported 8 and 10 rays, respectively, for these fins); pectorals 12 or 13; pelvics 5 or 6; caudal 13 or 14 (11 or 12 branched) in 3, the counts questionable since the outer rays are broken. Scales in lateral series (from shoulder girdle to caudal base) 30. Vertebrae (including urostyle) 13 + 19 = 32 in all (as determined by X-ray photography and further verified on the specimen dissected by Garman). Gillrakers (including all rudiments) on first arch 13 in 3 and 14 in one. Mandibular pores 2–2 in all; preopercular pores 7–7 in 2, the number indefinite in the remainder due to irregularities in the closing of the canal; pores on prcorbital represented by an open groove; no pores on top of head, but there is a pair of more or less developed longitudinal grooves on the interorbital and a nearly transverse postorbital canal which may be open or roofed over, with a pore at either end.

The dentition, of major importance in the classification of the species of *Poeciliopsis,* is essentially as described and figured by Garman. The outer teeth are moderately long, and are hooked backward and semi-erect throughout. The outer edge of each tooth is moderately expanded toward the tip, the inner edge broadly expanded and more or less angulate, sometimes approaching a secondary cusp. As a result each tooth has a rather asymmetrical leaf shape. The outer teeth are rather firmly implanted, especially as compared with the loosely attached teeth of such species as *P. turrubarensis* (Meek) and *P. presidionis* (Jordan & Culver). These teeth form a rather strongly curved and very even series, without a median indentation. They are comparatively large and few (about 20 to 24 in lower jaw). The inner teeth form an irregular series that lies close to and parallels the outer row. The inner row forms an even arc that extends farther backward in each jaw than the outer row, in an almost longitudinal continuation of the sweeping curve. The inner teeth slightly approach the outer ones in individual form.

There are about 8 or 9 narrow, dark, transverse bars, counting a rather indefinite small one at the base of the caudal fin and a trace of another at the shoulder. The better-developed bars are somewhat higher than the length of the eye. The next to the last is about as well developed below as above the midline of the side or is even better developed below, but proceeding forward the bars are more dorsal and some of those on the trunk reach well toward and sometimes to the mid-dorsal line. There is a fine dotted axial streak that disappears near the head, a fine pencilled black streak between the anal and caudal fins, and a weak, deep-lying, irregular row of dots between the caudal and dorsal fins and before the dorsal, disappearing anteriorly. Near the back the surfaces of the scale pockets become rather thickly and evenly peppered with black dots and are bordered by broad crescents, but downward on the sides the crescents become narrower and less regular. Some melanophores extend downward to the anal fin, but the lower part of the peduncle (except for the streak) and most of the abdomen are largely devoid of melanin. Melanophores very weakly line the dorsal, caudal and anal rays. There are rows of seattered dots along the pectoral rays but apparently none along the pelvic rays. No definite spots or bars are evident on any of the fins, which were described by Garman only as "clouded with brownish." The whole top of the head is pigmented, with some intensification on the snout, especially in a large blotch before each nostril. The upper lip is dark. The chin, including the intergular region, is puncticulate; the preorbital largely so. A narrow band of pigment also borders the orbit posteriorly but fades out ventrally. The cheek has almost no pigment. The opercles are silvery, with very little black pigment except for a definite concentration on the upper anterior part.

The axis of the body is almost straight and the dorsal and ventral contours are about equally curved anteriorly (suggesting a midwater habitat). The origin of the dorsal fin lies a little in advance of or approximately over the end of the anal base, and before the base of the caudal a distance measuring 1.7 to 1.75 times in the predorsal length (stepped measurements are used throughout). The origin of the anal fin lies

[2] Since this paper was put in type, specimens have been received that extend the range of *P. latidens* 35 miles farther south.

midway between the caudal base and the upper end of the preopercle. The distance from the tip of the snout to the insertion of the pelvic fin is contained 2.0 to 2.5 times in the standard length. The body cavity does not extend into the urosome.

The greatest body depth is contained 4.3 to 4.7 times in the standard length. The depth is maintained through the caudal peduncle, the least depth of which enters the head 1.6 to 1.9 times (the long preservation and poor condition of the specimens has probably resulted, however, in a deepening of the peduncle). The length of the head enters the standard length 3.7 to 3.9 times. When stepped into the head length, the depth of the head measures 1.3 to 1.4; the width, 1.5 to 1.6; the interorbital width, 2.3 to 2.4; the distance between the orbits ventrally, 2.6 to 2.8; the length of the snout, 3.6 to 4.8; the diameter of the eye, 3.6 to 4.4; and the greatest overall width of the mouth, approximately 3.0.

In top view the mouth is moderately curved. The midline length of the upper lip is about one-fourth or one-fifth its greatest width. In side view the gape is moderately developed; the length in projection is about equal to the diameter of the pupil. The main part of the gape forms an angle of about 20° with the horizontal throughout about two-thirds of its length and then becomes nearly vertical. The length of the upper jaw enters the head length 3.3 to 3.4 times. In side view the angle of the head is 33° to 39°; that of the muzzle 89° to 90°; in front view, lines across the orbit meet below at 15° to 19°.

The depressed length of the rounded dorsal fin is contained 3.7 to 4.0 times in the predorsal length. The anal fin is also rounded and its length enters the distance from its origin to the caudal base 2.2 to 2.4 times. The length of the pectoral enters the head length 1.5 to 1.6 times. The short pelvic reaches to about the middle of the anus and its length is contained about 2.6 to 3.0 times in the head length. The pelvics are separated by a space nearly twice the width of the base of either fin.

Diagnostic Characters of Poeciliopsis latidens (Other Than Those of Gonopodium)

A small, banded and/or spotted species of *Poeciliopsis* with the outer teeth few, asymmetrical, rather long and firm, hooked backward, and semi-erect throughout, arranged in a broad and evenly curved arc, without median indentation. Each outer tooth has a rather asymmetrical leaf shape (Garman, 1895, Pl. V, Fig. 11). The inner teeth are irregularly arranged, usually in one series, in an even arc that parallels the outer row of teeth, but is continued farther back, almost longitudinally, in each jaw. In form the inner teeth slightly approach the outer ones. There are about 7 to 12 narrow, dark transverse bars and spots in adult females, most commonly 8 or 9 (all faint spots or bars were included in the count). Typically at least one, and usually several, of the bars reach or nearly reach the mid-dorsal line. The bars and spots are variable in shape and irregular in spacing, both individually and bilaterally. Typically, few bars extend much, if any, below the middle of the sides. In the adult males, the spots and bars are usually fewer than in the females, but are often more intense; they vary from about 6 to 10, typically 7 or 8. About 1 to 3 bars extend to or close to the mid-dorsal line. As in the females, few bars extend below the middle of the sides and their development and spacing is variable and asymmetrical. There is a definite though not pronounced dark streak on the anterior dorsal ray and first inter-radial membrane in each sex. In the immature fish the bars and spots are usually less numerous and the bars often extend below the midsides. The preorbital is naked. The pores of the lateral-line system on the head are arranged as follows: mandibular pores well developed, 2–2; preopercular pores variable, 5 to 7, typically 7–7; and preorbital pores varying from an open groove (0–0) to 3–3. The body axis is almost straight and the dorsal and ventral contours are about equally curved anteriorly.

Gonopodium of Poeciliopsis latidens

Value of Gonopodial Characters.—The pertinence of *Glaridodon latidens* to the genus *Poeciliopsis* is attested by the remarkably constant finer structure of the marvellously complex gonopodium (the anal fin of the male modified as an intromittent organ). Very minor differences come to light when the organ of *P. latidens* is compared in detail with that of *P. presidionis* (the type species) and other species referred to the genus, but many trenchant distinctions become apparent when the gonopodium of *P. latidens* is contrasted with that of the species classed in any other genus of the subfamily Poeciliopsinae (Hubbs, 1924, pp. 9-10; 1926, pp. 62-64; 1936, pp. 232-235). These conclusions would have been evident on the basis of the gonopodial characters described in 1936 and are definitely strengthened and extended by a survey of the male genitalial characters of the Poeciliopsinae recently made by the senior author. There is scarcely a part of the gonopodium that does not exhibit fundamental resemblances within the genus *Poeciliopsis*, and striking differences between the related genera.

In this as in other groups of the Poeciliidae, the gonopodial characters are of vastly greater taxonomic significance, above the species rank, than all other known morphological features combined. For these reasons, the description of the gonopodium needs to be long and detailed, merely to define characters by which other species and genera differ.

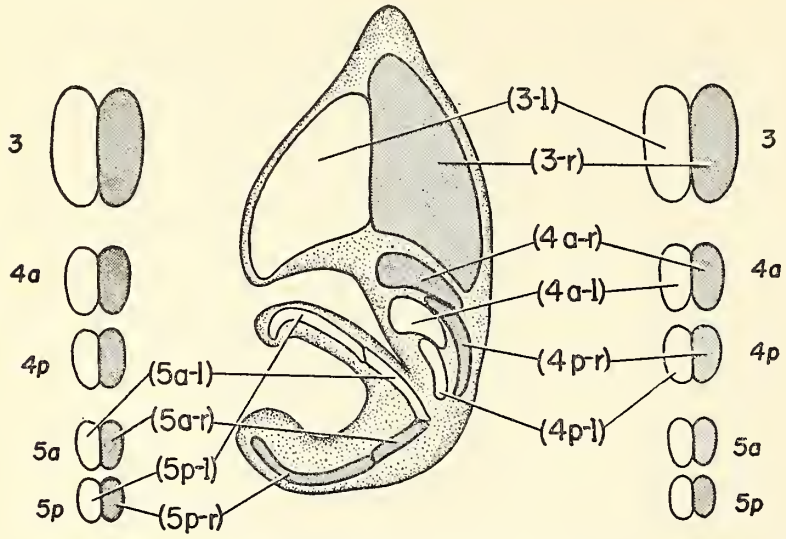
Methods of Study.—The gonopodial features are difficult to determine, because the rays are crowded and folded together, are in places bilaterally asymmetrical to a surprising and on first study confusing degree, and are highly modified, especially in the minute segments near the tips of rays 3 to 5. With repeated study and practice, however, the structures can be made out under appropriate lighting and magnification, particularly when the fin is manipulated with two fine needles. It is helpful, especially on early study, to tease (or chemically dissolve) the individual rays apart, or at least to unfold the fin or to pry the overlapping rays aside as the organ is turned, so that individual rays may then be followed. Toward the tip, where the segments become inordinately small, the manipulation entails great but, with care and patience, not insuperable difficulty. After the detailed structure is learned, the extreme constancy of the various parts can be perceived by gross examination and probing, without continued splitting apart of the rays. Earlier descriptions of the poeciliopsine gonopodia, such as those of Hubbs (1926), were crude and in some respects inaccurate, as was later pointed out (Hubbs, 1936).

Terminology.—In matters of orientation and terminology of parts we do not follow all the innovations made by Gordon & Rosen (1951) and Rosen & Gordon (1953). The gonopodium as a whole and its constituent rays are described as though held vertically downward from the base, as is customarily done in describing anal fins. Thus the front or anterior edge of the whole gonopodium or of any ray, in our terminology, is the morphological front part, not the lower or ventral part as usually described by Gordon & Rosen, who viewed the gonopodium as it is held at rest, extending almost straight backward. Similarly, we speak of the direction toward the tip of the fin as downward, not backward. The terminology of parts we follow agrees with the conventional illustration of the gonopodia in vertical position and avoids inconsistencies and awkward expressions. Similarly, we follow the primitive morphological orientation of rays that become rotated 90° or 180° in the elaboration of the gonopodium. In details, we retain the term *trough* rather than *canoe-like form* for the concave posterior edge of ray 5; *crescentic horn* for a structure curving over the extreme tips of the rays; and *membranous hood* for the dermal keel around the tip of the gonopodium. And we do not carry over from the nonhomologous condition in *Xiphophorus* the distinction between proximal and distal serrae (on ray 4p). We find it convenient to distinguish formally between the left and the right halves of given rays or branches of rays, for the two halves are often structurally different; thus, 3-l means the left side or segments of ray 3, and 4p-r means the right side or segments of the posterior branch of ray 4. Since the modification of the anal fin involves almost all its parts, the entire fin, beginning with rudimentary rays 1 and 2, is treated as the gonopodium. The last ray, as we consistently define it for the soft dorsal and anal fins, comprises the last two elements that are structurally distinct, including their structural bases, whether these elements be approximated or widely separated.

Description of Gonopodium.—The following description is based on the 57 males with completely or almost completely elaborated gonopodia in a large collection (UMMZ No. 164642) from Rio Yecorato, tributary of Rio Sinaloa, about 7 miles north of Guasave, Sinaloa, Mexico; collected by R. G. Miller on May 6, 1942.

The gonopodium is a very long and slender structure, inserted far forward (preanal length 43 to 48, averaging 46, percent of the standard length, in 30 specimens) but extending to within about an eye's length of the procurrent caudal rays. In the same specimens the overall length is 42 to 47 (average 45) percent of the standard length. The gonopodium is relatively thick (weakly compressed); near the middle of its length its left—right width is more than half the anteroposterior length.

The main rays (3 to 5) of the gonopodium are profoundly modified in size, shape, relative position and symmetry, as is indicated in part by the somewhat diagrammatic sketch (Text-fig. 1) representing the approximate appearance of a cross-section toward the middle of the length of the longest rays. The right side is weakly convex, the left side deeply concave, and ray 5 is rotated clockwise so that the tips of 3-l and 5p-l are almost in contact. As in all of the hundreds of specimens of *Poeciliopsis* examined, the tube is consistently on the left side. All 57 males in the series studied and 7 additional more or less completely transformed specimens have a sinistral gonopodium. This finding confirms the views of Hubbs (1936: 232-235) and of Hubbs & Hubbs (1945: 290-293), that the laterality of the gonopodium is consistently characteristic of the genus in all Poeciliopsinae (other than *Xenophallus*). The recent re-exam-



TEXT-FIG. 1. Semidiagrammatic cross-section of the gonopodium of *Poeciliopsis latidens*, toward the middle of the length of the longest rays, with the rays and their branches and bilateral halves identified and homologized with the corresponding parts of a more primitive gonopodium (diagrammed at either side).

ination of the gonopodia in the various genera of the family reveals no other exception to this rule.

The asymmetry of the organ is consistent also in the position of the first two rays and of the posterior rays (behind 5), as is described below, and involves the detailed flexure of the main rod (composed of rays 3, 4 and 5). As viewed toward its anterior edge this rod, consistently, is gently curved longitudinally toward its right side, with the strongest point of flexure about opposite the tip of ray 6. Beyond this point the gonopodium curves very gently toward the left side of the fish, outward to the moderately thickened and opaque area, about five-sixths of the way out, beyond which the terminal region, rapidly narrowing and becoming glassy, swings back toward or to the midline. The extreme tip is hooked almost at a right angle forward and slightly to the left.

When the undissected gonopodium is viewed from the right side, beyond the little distorted base and before the highly modified tip, there are visible: (a), the strongly curved right side of ray 3 (3-r), occupying about half the side; (b), the part of 4a-r that is not overlapped by the produced edge of 3-r; (c), almost all of 4p-r; then, (d), curving away, 5a-r, and, (e), when the troughlike ray 5 is rotated counterclockwise away from its normally infolded position, also 5p-r, curving away toward the left. When the gonopodium is viewed from the left, there are seen only: (a), the extreme front of 3-r; (b), the whole rounded left side of 3-l (narrower and with a less trenchant rim than the right side of 3-r); and (c), the morphologically right (but now actually left) side of 5p-l and 5a-l, plus (d), the sloping left side of 5a-r and, (e), the very edge of 5p-r; also (f), some of the left side of 5p-r, when the trough is rotated counterclockwise. When in normal position 5-l, rotated nearly 180°, fits against or close to the left edges of 4a-r, 4a-l, and 4p-l; and 5-r is rotated nearly 90°, in the same direction.

The gonopodium is heavily charged with black pigment along the right side of ray 3. The posterior part of this ray is clear, except very near the base and except on the outer third of the fin, out to the unpigmented vitreous tip. There is also a heavy suffusion of pigment along the trough of ray 5 and there are scattered dots on the right side of the main part of the gonopodium and along the short posterior rays.

Rays 1 and 2 are more minute than in most related forms. They are consistently deflected somewhat toward the left side and lie in a shallow trough between the basal forks of 3. Ray 2 overlaps at most one segment of 3 beyond the unsegmented base.

Each rather compressed fork in the hidden base of *ray 3* is broadly expanded at the extreme end, beyond which the fork is at first slender and then is dilated anteroposteriorly toward the union of the forks; the expansion continues toward the basal segments. The unsegmented base is long. The ray is only moderately expanded, less so than in most related forms, along the first few segments beyond the unsegmented base. These expanded segments are only about twice as broad as long. The basal sutures of 3-r run transversely near the posterior edge, but are deflected slightly downward toward the front edge. The first few segments of 3-l are separated by V-shaped incisions on the posterior margin. The longitudinal suture between 3-l and 3-r runs distinctly to the left side of the anterior

crest of 3, farther to the left than in some other species of the genus. Throughout the length of ray 3 the sutures remain essentially transverse. Before the middle of the length of the ray, the segments change from broader than long to about square (as seen at right angles to the surface of the ray). Near the outer limit of the slight distal thickening, ray 3 narrows and in cross-section changes from a broad triangle to a relatively thin, weakly curved, transverse structure greatly narrowed in lateral view but only moderately constricted in anterior view. Beginning with a point where this transformation in form has become nearly completed, and extending outward for a distance about twice the length of one of the median segments of the ray, several segments are almost completely fused to form the *consolidated segments* characteristic of the genus. This structure is relatively soft. Beyond the consolidated segments, the sutures again become sharp and a few of the segments become subserrate on the right margin. These outer segments are somewhat wider than long but are not strikingly dilated (as they are in some species). Each segment-half (the two halves flare apart in one plane) is longer than wide. The extreme distalmost segments become greatly reduced, most notably in width. Somewhat longer than broad, they extend to the extreme hooked tip, on the inside of the curve.

Near the base *ray 4* is moderately thickened, more so on the anterior than on the posterior edge. The basal segments in side view are less than one-half broader than long. The left and right halves are in full and approximately symmetrical contact. At the very base ray 4 lies just behind 3, the two abutting without overlap, but soon 4 becomes displaced toward the right, so as to lie against 3-r and to be overlapped in part by the trenchant posterior edge of 3-r. Not far beyond the tip of 6, the ray branches to form 4a, which takes over the thickened portion and becomes rotated far toward the left, and 4p, which is somewhat broader in side view than 4a. Ray 4a becomes more nearly transverse than longitudinal, whereas 4p remains chiefly longitudinal. As a result the whole ray is very strongly curved. Its total anteroposterior width is much less than the width of the left side of 3. All parts of 4 are comparatively robust. In cross-section each half of 4p is a thin plate, gently concave on the left side and convex on the right. The left segments of each branch come to lie partly behind their right partners (as is particularly evident when the rays are manipulated with a needle), because the thickened anterior (now essentially left) edge of 4a-l lies behind 4a-r. In side view, both branches of 4-l are narrower than those of 4-r. The posterior (now largely right) edges of 4a-l and 4a-r somewhat overlap 4p-l and 4p-r, respectively. The segments of 4, like those of 3, remain essentially transverse. Out toward the modified tip, the segments of 4p become nearly twice as long as broad; those of 4a, at least twice as long as broad. In this region the segments of 4a are about as long as those of 3 and about one-fourth longer than those of 4p.

Opposite the consolidated segments of ray 3, *ray 4a* flattens out transversely so as to lie close against and directly behind the similarly flat and transverse part of 3, and its segments are similarly though less completely consolidated. As the consolidated segments are approached, 4a-l becomes rather narrow, less robust, and very much softer than 4a-r, which is here the principal element. Toward the extreme tip, the segments of 4a-r, separating 3 and 4a-l and remaining transverse, become somewhat dilated but remain less than twice as wide as long. Continually diminishing in size, the segments of 4a extend to the extreme tip, hooking around the curved tip of 3. In the terminal region of the gonopodium, 3 and 4a are so closely apposed and are so similar (and are so different from and so well separated from 4p) as to give the false impression that they are the anterior and posterior branches of the same ray.

In the thickened subterminal section of the gonopodium, the segments of *ray 4p* become transformed into serrae. Including two or three segments of transitional form, the serrae number about 17 on each side (left and right), and cover about one-fifth the total length of the gonopodium. All but the most basal serrae of 4p-r are like rose thorns, each arising from a base about twice as long as broad. Each thorn is directed essentially backward, with some angulation toward the left. The serrae of 4p-l, in contrast, are directed toward the left. The several distalmost serrae of 4p-l form thorns that are wider and more compressed than the opposite elements of 4p-r. The other serrae of 4p-l are strikingly unlike the thorns of 4p-r, being flat and more or less two-horned, or merely truncated. In this outstandingly peculiar bilateral asymmetry of the serrae of 4p, *Poeciliopsis latidens* agrees with other species of its genus but differs from those of related genera. The serrae extend to about opposite the end of the consolidated segments of 3 and 4a. The more basal and the more distal serrae of the two sides are readily separated by a fine needle, and those of each branch are very loosely articulated, but the more median ones are more firmly connected (bilaterally and longitudinally). Toward the tip, ray 4p swings onto the left side of the gonopodium. Beyond the serrae the segments become

extremely minute but seem to continue onto at least the base of the abruptly curved extreme tip of the organ. Such features are seen only under high magnification, with the gonopodial tip turned at the most propitious angle.

At its extreme, essentially symmetrical base, *ray 5* is considerably strengthened, though much slenderer than it is farther out. Very near the base, the ray rotates toward the left so that only the anterior edge lies on the right side. Before the end of ray 6, the left edge has already become trenchant and turned clockwise nearly 180°. Here is seen the start of the trough that characterizes the species of *Poeciliopsis* and some of the related genera. This trough is formed by the flaring apart, to somewhat less than 90°, of the halves (5-l and 5-r) of the ray. In this trough, as already noted, 5-l normally lies against 4, with its tip close to the posterior edge of 3-l. Throughout most of the length of the gonopodium 5a-l and 5a-r remain connected only at their anterior edges, while 5p-l and 5p-r are widely separated. Consequently, the trough is floored by the two halves of 5a as well as 5p. The whole trough is capable of considerable rotation: it can be moved clockwise to nearly close the gonopodial tube, or counterclockwise, tending to restore the primitive linear sequence of the rays. Near the middle of the gonopodium and for some distance toward the base, the sides of the trough diverge at nearly a right angle, but both basad and apicad the halves approach one another to decrease the angle. Throughout most of the length of the ray the segments of both 5a and 5p are greatly compressed platelets. The relative shapes of these segments differ at different levels and on the two sides. Just beyond the tip of 6, the left side is much more expanded than the right. The posterior rim of 5p-r rather abruptly swings toward the left and somewhat forward, and the right side rapidly regains a width approximating that of the left. The proportionate width of the segments of the two branches is dissimilar, however, for 5a-l as seen from the trough is little wider than 5p, whereas 5p-r is about twice as wide as 5a-r. In cross-section 5a-r is scarcely curved. Near the middle of the gonopodium the segments of ray 5, considerably shorter than those of 3 or 4, differ in dimensions in the two branches and on the two sides: those of 5a average about one-fourth longer than those of 5p; those of 5a-l are about twice as long as broad, whereas those of 5a-r are about 2.5 times a long as broad; those of 5p-l average about one-half longer than broad, whereas those of 5p-r are approximately square. Throughout their lengths, from the extreme base to the tip, the sutures of both sides and of both branches remain essentially transverse, and the two free edges form a rather even line, unmodified in the direction of serrae, lobes, or other processes. Each edge, more particularly the left, is hooked inward.

The *outer segments of ray 5* are considerably modified. Opposite the most basal serrae of 4p, the squarish segments of 5a-l have become about three times as broad as the more rodlike and strongly curved elements of 5p-l, and the segments of 5a-r are also broader than those of 5p-r. Beyond the middle of the gonopodium the stiff free edge of 5-l remains trenchant and approximated to the left posterior edge of 3, whereas 5-r becomes narrower and softer. Opposite the basal serrae ray 5-r becomes membranous and seems to disappear, so that at the tip of the gonopodium only 5-l is recognizable. Here 5-l forms a thin keel along the side of 4, just in advance of 4p-l, which is similarly turned toward the left. Opposite the base of the consolidated segments (and opposite the place where the bicornute serrae of 4p-l change into flat thorns), the posterior edge of 5-l remains nearly straight while the anterior edge is sharply contracted, so as to decrease the width of 5-l abruptly and greatly. Opposite the distalmost serrae 5-l becomes an extremely fine and delicate double strand closely connected with the almost similarly reduced tip of 4p. Both tips run along the edge of a slightly expanded membrane that narrows down to the left edge of 4a, near the base of the apical hook. In some specimens, on close scrutiny, the now excessively attenuated strands may be followed, together, along the edge of the hook.

All segments in the *tip of the gonopodium* are relatively soft. The extreme tip is minutely hooked, forward and somewhat to the left, nearly to a right angle. Along the outer edge of the hook the apex bears a thin, evenly rounded *membranous hood* about one-third as wide as long (the length measured along the morphologically posterior but here transverse apex). The weak to obsolete crescentic horn runs in the base of the membranous hood along the outer curve of the terminal segments of 3 and 4, which reach to the extreme tip.

The *four posterior rays* of the gonopodium are extremely short. Of these only ray 6 is thickened. It is in a thick opaque fleshy mass that is deflected consistently onto the left side of 5. The swollen tip of 6 is rounded along the front edge, both transversely and longitudinally. It is somewhat more convex on its left than on its right side. The segments become fused in the outer two-thirds of the swollen area.

Rays 7 and 8 are also distinctively modified, but in a very different and peculiar way. Both remain very slender throughout, not developing

swollen tips such as are characteristic of certain other genera of Poeciliopsinae (7 may be very slightly strengthened near its tip). Each is only incipiently branched at the tip. These rays originate apart, in normal file, but instead of remaining well separated throughout, as they do in the young males and in females of all ages, and as they do in the males of most poeciliids, they abruptly converge to become very closely approximated or in actual contact along the middle section of their lengths. Here ray 7 consistently lies more or less to the left of 8, giving rise to the false impression that these rays are the slightly dislocated left and right halves of a single ray. Distally the rays diverge rather widely. This peculiar condition of 7 and 8 is a consistent character of *P. latidens* and of the other species of *Poeciliopsis*. It holds for all 57 developed males in the series studied, with one abnormal exception. In this aberrant specimen, ray 6 is minute and clavate and is concealed in the inter-radial membrane just in advance of the base of 7. The latter ray has taken over the form of 6, presumably because the developmental field that normally affects 6 acted on 7, which in this specimen follows 5. In this aberrant gonopodium, 8 is less slender than usual and is well separated from 7 and from 9.

The fore and aft elements of *ray 9* are greatly reduced. In some specimens they are discernible with difficulty, even under high magnification and good illumination, but are probably never completely atrophied. They lie close together well behind 7 and 8, often in a more or less thickened and irregular little mass or lobe.

A small scaleless area surrounds the posterior end of the gonopodial base, but there is no naked groove behind the fin.

The multitudinous gonopodial characters described above are all definitive—of the subfamily, genus, or species. These observations confirm the opinion that no structure-complex in fishes is known to be more replete with characters than is the gonopodium.

Comparison between Gonopodia of P. latidens and P. presidionis

When the gonopodium of *Poeciliopsis latidens,* as described above, is compared in detail with that of topotypes of *Poecilia presidionis* Jordan & Culver, the type species of *Poeciliopsis,* a most remarkable agreement in fundamental structure is seen to prevail, from the first ray to the last and from the base of the fin to the highly modified tip. Many minor differences, however, appear, and are validated by studying series of specimens. These distinctions are mostly of degree and illustrate the range of differentiation to be found between well-defined species of the genus, all within the limits of a single highly distinctive general pattern.

Insertion of Gonopodium (measured from extreme base).—*P. latidens:* farther forward, preanal length 2.1 to 2.33 in standard length. *P. presidionis:* preanal length 2.0 to 2.1.

Length of Gonopodium (from origin at extreme base to farthest tip).—*P. latidens:* averaging longer, 2.1 to 2.4 in standard length. *P. presidionis:* 2.3 to 2.5.

General Dimensions of Gonopodium (near middle).—*P. latidens:* relatively thicker (left-right width more than half anteroposterior dimension). *P. presidionis:* more compressed (width less than half anteroposterior length).

First Few Segments of 3-r (beyond unsegmented base).—*P. latidens:* less expanded, about twice as broad as long, with sutures only slightly decurved forward and downward; without longitudinal depression. *P. presidionis:* more expanded, about thrice as broad as long, with sutures strongly oblique (forward and downward); depressed longitudinally to receive overlap of 4.

Longitudinal Suture between 3-l and 3-r.—*P. latidens:* displaced a little farther toward midline. *P. presidionis:* nearer front ridge.

Width of Ray 3 near Middle of Fin.—*P. latidens:* about half width of gonopodium at same level. *P. presidionis:* much less than half width of gonopodium.

Consolidated Segment of 3.—*P. latidens:* very soft, narrower, with both contours grading evenly into those of following rays. *P. presidionis:* stiff, broader, with keeled margin at left side abruptly constricted just before end (but right edge grading evenly into edge of following segments).

Segments of 3 beyond Consolidated Segment.—*P. latidens:* each segment-half (the two halves flaring apart in one plane) longer than wide; a few subserrate on the right margin. *P. presidionis:* each segment-half about as wide as long, rather irregular in form but not distinctly subserrate on right margin.

Basal Part of 4 (opposite middle part of 6).—*P. latidens:* scarcely dilated; segments nearly square, less than 1.5 times broader than long; abutting 3-r. *P. presidionis:* markedly dilated; segments about twice as broad as long; overlapping 3-r.

Ray 4 near Middle of Length.—*P. latidens:* 4a more nearly transverse than longitudinal; ray much more curved; its total anteroposterior width much less than width of left side of 3; all parts more robust; 4-l normally lying largely behind 4-r. *P. presidionis:* 4a more nearly longitudinal than transverse; ray much straighter; its total width about equal to width of left side

of 3; all parts more compressed and fragile; 4-l normally lying beside 4-r.

Broadest Subterminal Segment of 4a (about midway between consolidated segment and tip of ray).—*P. latidens:* definitely less than twice as broad as long. *P. presidionis:* twice or somewhat more than twice as broad as long.

Trough of 5, Basal to and near Middle of Gonopodium.—P. latidens: less flaring, the sides forming an angle of less than 90°. *P. presidionis:* usually flaring more than 90°.

Relative Widths of Segments of 5 (relative lengths in cross-section, as in Text-fig. 1).—*P. latidens:* 5a-l little wider than 5p-l as seen from trough; 5a-r about one-half width of 5p-r. *P. presidionis:* 5a-l about twice width of 5p-l; 5a-r and 5p-r subequal.

Curvature of 5a-r (in cross-section).—*P. latidens:* scarcely curved. *P. presidionis:* strongly curved.

Several Segments of 5a-l Basad from Consolidated Segment of 3.—P. latidens: With morphologically anterior edge (now posterior and flexed to the left) forming a straight line. *P. presidionis:* each segment with anterior (now posterior) edge produced toward left into a firm rounded lobe.

All Ray Tips.—P. latidens: very soft. *P. presidionis:* stiffer.

Extreme Flexed Tip of Gonopodium (twisted forward and to left).—*P. latidens:* curved nearly to right angle or hooked beyond a right angle. *P. presidionis:* not curved to a right angle.

Tips of Rays 3 and 4a in Flexed Tip of Gonopodium.—P. latidens: extending to extreme tip; inner edge of apical flexure not appearing keeled. *P. presidionis:* ending abruptly in terminal membrane well in advance of tip, usually not far beyond middle of final flexure, so that the hood seems to extend around tip onto inner side of curve.

Crescentic Horn (in flexed tip of gonopodium).—*P. latidens:* weak to obsolete, not extending to tips of rays 3 and 4a. *P. presidionis*: usually strong and conspicuous, occasionally rather weak; extending in a weak hook well beyond tips of rays 3 and 4a.

Comparison with Poeciliopsis fasciata

Poeciliopsis latidens is most closely related to *P. fasciata* (Meek), from which it differs primarily in coloration and in markings (Table I). The principal difference lies in the number and shape of the vertical bars, which show sexual dimorphism in each species. Mature males of *fasciata* typically have only 3 to 5 narrow, vertical bars, and the females usually have 4 to 5; the total variation is from 2 to 6. Mature males of *latidens* usually have 7 to 10 broader bars and spots (or spots only), and the females generally have 8 to 12 such markings; the total range is from 5 to 14.

There is considerable variation in the markings of *P. latidens*. For example, in a sample from the Rio Quelite, Sinaloa (UMMZ No. 160576), vertical bars are almost absent and there are fewer spots than usual, 5 to 9, usually 6 to 8, in females, 7 each in two males, and 5 only in one male. In a collection from the Rio Piaxtla (about 45 miles north of Mazatlan), the vertical bars are narrow as in *fasciata,* but there is no dark streak at the front of the dorsal fin and the bars are more numerous than they are in that species. The markings of *latidens* do not appear to be constant within a single stream system, but all samples examined (25) can be readily distinguished from *fasciata* on the basis of one or more of the characters listed in Table I, and on other less obvious but constant differences, chiefly of pigmentation.

There appears to be a broad gap in the ranges of the two species. *P. latidens* is not known south of Mazatlan[3] and *P. fasciata* has not been recorded north of the Isthmus of Tehuantepec. The known range of *latidens* is believed to represent the actual range reliably because the region south of Mazatlan has been visited by fish collectors and no species of *Poeciliopsis* corresponding to *latidens* has been obtained. On the other hand, the area immediately to the west of the basin of the Rio Tehuantepec (where *fasciata* lives) is little known and we have seen no collections between western Oaxaca and Acapulco, Guerrero. In the brackish lagoon about 10 miles north of that city (Laguna Coyuca, UMMZ No. 164689) there is a species of *Poeciliopsis* that is representative of (and perhaps identical with) *fasciata;* the same species also has been taken in Arroyo Nuxco, Guerrero (UMMZ No. 159896), about 60 miles northwest of Acapulco. It may eventually be found that *latidens* and *fasciata* are connected by a chain of intermediates in the area between Guerrero and Sinaloa. If so, the two forms, with, perhaps, some additional subspecies, may be referred to a single species (*latidens*).

Range

As now delimited, *Poeciliopsis latidens* is known to occur in streams tributary to the Pacific from the basin of the Rio del Fuerte, along and near the boundaries of Sonora, Sinaloa and Chihuahua, southward to the Rio Quelite (north of Mazatlan) and, along the coast, to the bay at Mazatlan, Sinaloa—all in northwestern Mexico.[3] The northernmost locality from which we have seen specimens is a tributary of the Rio

[3] See footnote on page 3.

TABLE I. COMPARISON BETWEEN TWO SPECIES OF *Poeciliopsis* FROM MEXICO
Data for *P. latidens* based on many specimens from throughout its known range

Character	*P. fasciata*	*P. latidens*
Vertical bars	Fewer, narrower, less variable in number, none replaced by spots.	More numerous, broader, very variable in number, some or all nearly replaced by spots (occasionally obsolescent).
Lateral markings: In mature males	2 to 6, typically 3 to 5, bars[1], 50 percent or more extending well below midsides. Two or more bars usually meet or nearly meet at midline of back on each side.	5 to 12, usually 7 to 10, bars and spots (or spots alone), the bars rarely extending well below midsides. Usually at least one, occasionally two, bars meet at midline of back in well-barred populations.
In adult females	3 to 6, usually 4 or 5, bars[2]. At least one and usually two or more bars meet at mid-dorsal line on each side.	7 to 14, usually 8 to 12, bars and spots (or spots alone). Typically, one bar reaches to or nearly to mid-dorsal line on one side (in well-barred forms).
Front of dorsal fin	Typically with a jet-black streak on first two dorsal rays and inter-radial membranes; concentrated at base of fin as a black spot.	First ray and inter-radial membrane with a dark streak, not concentrated to form a spot; streak often very weak and pigment nearly absent on membrane in some populations.[3]
Front of anal fin: In mature males	Blackened at base of gonopodium; outer parts of rays 3 and 5 not notably darkened.	No black spot at base of gonopodium; outer parts of rays 3 and 5 conspicuously dusky.
In adult females	Typically with a black spot or dash.	A dark streak weakly developed or absent.[3]

[1] Based on 58 male subtopotypes (UMMZ No. 161517).
[2] Based on 23 female paratypes (CNHM No. 4716).
[3] Occasional individuals in some populations approach *fasciata* in this character.

del Fuerte 7 miles northeast of Alamos, Sonora (UMMZ No. 161554). About 25 collections of this species are deposited in the Museum of Zoology, University of Michigan, and others at the University of California (Los Angeles) have been studied.

LITERATURE CITED

ALVAREZ, JOSÉ

1950. Claves para la determinación de especies en los peces de las aguas continentales Mexicanas. Secretaría de Marina, Dirección General de Pesca e Industrias Connexas, México, pp. 1-144, figs. 1-16.

DE BUEN, FERNANDO

1947. Investigaciones sobre ictiológia Mexicana. I. Catálogo de los peces de la región Neártica en suelo Mexicano. Anales Inst. Biol., 18 (1): 257-348, 9 maps.

GARMAN, SAMUEL

1895. The Cyprinodonts. Mem. Mus. Comp. Zool. Harvard Coll., 19 (1): 1-179, pls. 1-12.

GORDON, MYRON, & DONN ERIC ROSEN

1951. Genetics of species differences in the morphology of the male genitalia of xiphophorin fishes. Bull. Amer. Mus. Nat. Hist., 95: 409-464, figs. 1-23, pls. 17-18.

HUBBS, CARL LEAVITT

1924. Studies of the fishes of the order Cyprinodontes. I-IV. Misc. Publ. Mus. Zool. Univ. Mich., No. 13: 1-31, pls. 1-4.

1926. Studies of the fishes of the order Cyprinodontes. VI. Materials for a revision of the American genera and species. Misc. Publ. Mus. Zool. Univ. Mich., No. 16: 1-87, pls. 1-4.

1936. Fishes of the Yucatan Peninsula. Carnegie Inst. Wash. Publ. No. 457: 157-287, pls. 1-15, fig. 1.

HUBBS, CARL LEAVITT, & LAURA CLARK HUBBS

1945. Bilateral asymmetry and bilateral variation in fishes. Papers Mich. Acad. Sci., Arts, and Letters, 30 (1944): 229-310, figs. 1-2, pl. 1.

Jordan, David Starr, Barton Warren Evermann & Howard Walton Clark

1930. Check list of the fishes and fishlike vertebrates of North and Middle America north of the northern boundary of Venezuela and Colombia. Rept. U. S. Comm. Fish., 1928, pt. 2: I-IV, 1-670.

Meek, Seth Eugene

1904. The fresh-water fishes of Mexico north of the Isthmus of Tehuantepec. Field Col. Mus., Publ. 93 (Zool. Ser.), 5: i-lxiii, 1-252, figs. 1-72, pls. 1-17, 1 map.

Regan, Charles Tate

1906-08. Pisces. *In:* Biologia Centrali-Americana, pp. i-xxxiii, 1-203, 7 maps, pls. 1-26.

1913. A revision of the Cyprinodont fishes of the subfamily Poeciliinae. Proc. Zool. Soc. London, 1913, pp. 977-1018, figs. 168-173, pls. 99-101.

Rosen, Donn Eric, & Myron Gordon

1953. Functional anatomy and evolution of male genitalia in poeciliid fishes. Zoologica, 38 (1): 1-47, text-figs. 1-47, pls. 1-4.

EXPLANATION OF THE PLATE

Plate I

Fig. 1. Adult males of two species of *Poeciliopsis*. Above: *P. latidens*, 21.2 mm standard length, from Rio Yecorato, Sinaloa, Mexico (UMMZ 164642). Below: *P. fasciata*, 22.2 mm long, from Rio Tehuantepec, Oaxaca, Mexico (UMMZ 161517).

Fig. 2. Adult females of two species of *Poeciliopsis*. Above: *P. latidens*, 28.5 mm long, same data as Fig. 1. Below: *P. fasciata*, 28.0 mm long, same data as Fig. 1.

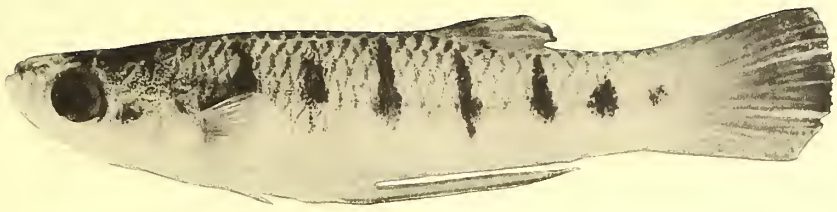


FIG. 1

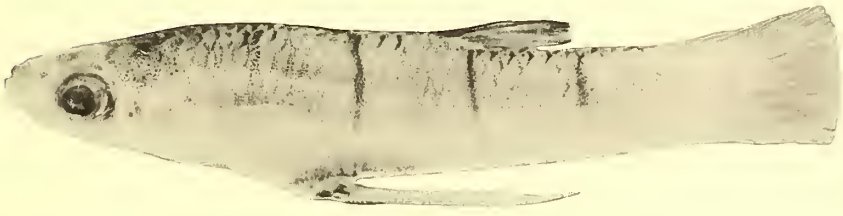


FIG. 2

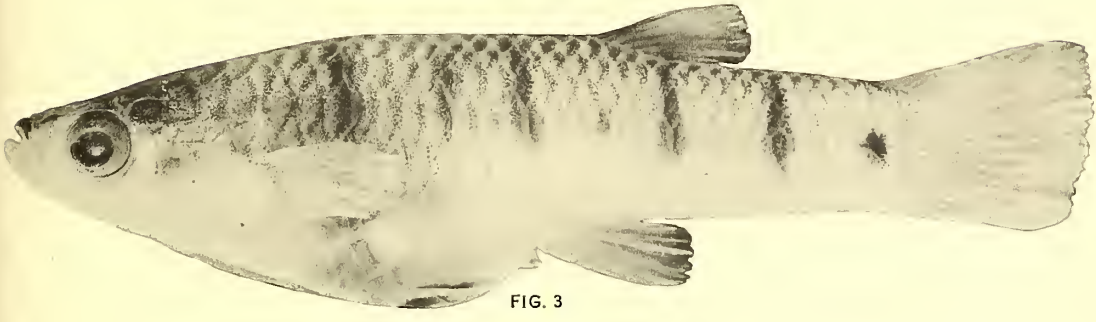


FIG. 3

FIG. 4

GLARIDODON LATIDENS, FROM NORTHWESTERN MEXICO,
REDESCRIBED AND REFERRED TO POECILIOPSIS

Under EXPLANATION OF THE PLATE opposite:
FIG. 1 refers to figs. 1 and 2 on this plate
FIG. 2 refers to figs. 3 and 4 on this plate

2

A Second Case of Survival by a Teleost without a lower Jaw

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(Plate I; Text-figure 1)

THE case of a fish, *Anoptichthys jordani* Hubbs & Innes, which had survived without a lower jaw, was described by Breder (1945). While the cause of this loss was not at all clear, it was speculated that it probably was of genetic origin rather than some accidental damage, on a basis of the unlikelihood of recovery from such an injury. Nothing further was found on this subject until a fish showing a similar defect was discovered in a tank of *Astyanax mexicanus* (Filippi). It was first noticed on about October 1, 1952, but it was not until later that it was realized that the fish was beginning to resemble the case noted above. The photographs of the living fish, Plate I, were taken on December 17, 1952. The resemblance to the condition of the cave fish earlier described is unmistakable. The teeth of the upper jaw may be best seen in the front view and the protruding glossohyal and urohyal are evident in the lateral view. The "mouth" was a vertical slit similar to that of the cave fish, *Anoptichthys,* and feeding was seen to be done in a similar manner. The photograph in the lower right of Plate I shows the fish about to pick a piece of dry fish-food from the bottom. It would seem certain that precisely the same osseous elements had disappeared in both cases, which led to the development of identical feeding methods. Although this fish was rather thin it fed readily and managed to engulf what seemed to be the usual amount for this species.

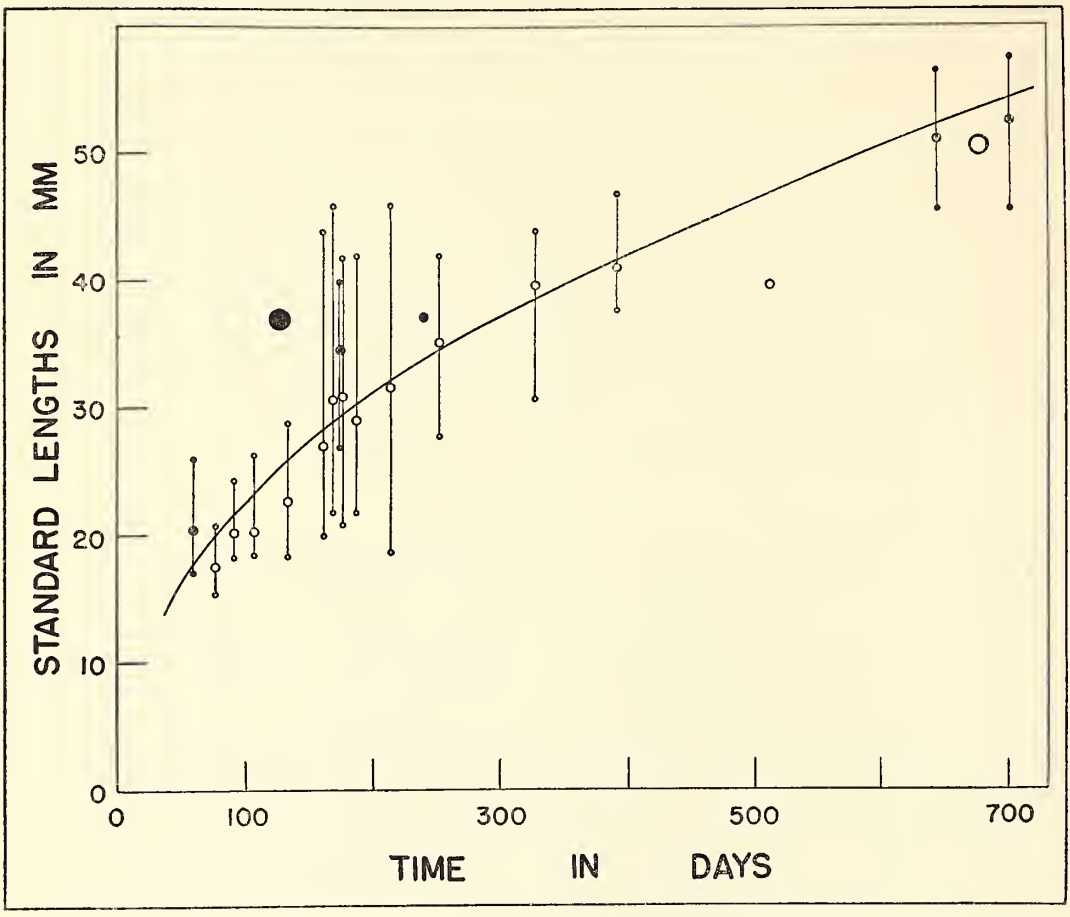
Since the fish showed the secondary sex character of hooks on the anal fin it was established in a well-planted aquarium with a female of its own size which had previously been known to spawn, in the hopes that it might reproduce. Although the two fish remained in each other's company for most of the time, after the manner of this species, there was no indication of any reproductive activity at any time.

From this time on the fish seemed to lose ground and apparently had increasing difficulty in obtaining food. Its emaciation increased and when evidently about to expire, it was preserved on April 13, 1953.

A radiograph of the head made shortly after the fish was fixed in formalin is shown in Plate I. This confirmed the earlier suspicion that the same elements had been lost by the fish as those described in the preceding report on a cave fish. The most notable difference is associated with the orbital series of bones which in this instance did not tend to crowd in about an empty socket, since they were held to their normal positions by the presence of an intact eye.

This fish lived 676 days, having been one of a brood from a spawning on June 7, 1951, at the fish cultural establishment of Mr. Albert Greenberg at Tampa, Florida. The cave fish, on the other hand, survived only about six months, circa 180 days. The behavior of the two was strikingly similar, both assuming a more nearly vertical position to obtain food from the bottom as they grew older, the position in both cases reaching nearly 90° in their later days.

Evidently this defect is carried in the genes of both the cave fish and their river-dwelling ancestors. Its appearance is thus clearly not linked to the depigmented and eyeless condition of the cave forms. Both cases developed from a small group of fishes which had been inbred to a considerable extent, which condition may have been responsible for the phenotypic appearance of this deficiency. The cave fish attained a growth of 37 mm standard length in a Tampa greenhouse while the river fish attained a standard length of 51 mm in the Department labora-



TEXT-FIG. 1. Growth of *Astyanax,* open circles, and of *Anoptichthys,* closed circles, as obtained in the laboratory. The smaller circles above and below connected by vertical lines represent the extreme range, the slightly larger circle between representing the means. The two large circles standing alone represent respectively the two jawless cases. There are represented on this graph 300 measurements of *Anoptichthys* and 535 measurements of *Astyanax.*

tory in New York. On dissection, normal but small and thin testes were found, which looked typical of fishes under conditions of undernourishment. It still remains unknown at this writing whether such fish do survive long enough and in sufficient vigor to reproduce.

In order to determine more accurately, if possible, what effect on the rate of growth this oral malformation exerted, measurements of age and size which had been accumulated in the laboratory for various experiments were draughted, which amounted to 300 cave fish from La Cueva Chica stock and 535 river fish of the stock on which the work in the laboratory has been carried out and which are descendents of the fish taken in Mexico as near to La Cueva Chica as practicable.

A growth curve calculated for the cave fish is represented by the following equation:

$$y = 5.007x.^{0.369}$$

and for the river fish the following:

$$y = 2.143x.^{0.501}$$

This difference is probably expressive of a slightly larger growth rate shown by the cave fish, but it is not great in any case. Because of the varied sources and times from which the data were accumulated, it may have little significance, although there is corollary evidence which indicates that there is a real difference. It would seem to be rooted in the fact that the cave fishes consume more food than the eyed river fish, the latter taking considerable notice of what transpires outside of their aquarium. For present purposes it is adequate and more satisfactory to handle all the data together, in which case the equation becomes

$$y = 2.841x.^{0.451}$$

which is the curve shown in Text-fig. 1. This

graph gives the growth as obtained in the laboratory for both cave fish and river fish and serves as a satisfactory base line to which these anomalies may be referred.

The jawless cave fish is clearly larger for its age than most of the cave fish from the laboratory. This fish spent its entire life in the Florida hatchery. There the temperatures average somewhat higher and the light is brighter and every effort is made to grow fish as fast as possible. This probably accounts for the difference, but nevertheless there is certainly no suggestion of any stunting associated with the abnormality.

The river fish which spent all but the first two months of its life in the laboratory is clearly close to the two mean values for normal fish indicated on either side of it. Here again there is no suggestion of stunting. It would thus appear that whatever condition developed in these two fishes which led to their death took place rather rapidly and was not a long protracted semi-starvation, as might be assumed were it not for the comparative data herewith submitted.

Reference

Breder, C. M., Jr.

1945. Compensating reactions to the loss of the lower jaw in a cave fish. Zoologica, vol. 30, no. 10, pp. 95-99.

EXPLANATION OF THE PLATE

Plate I

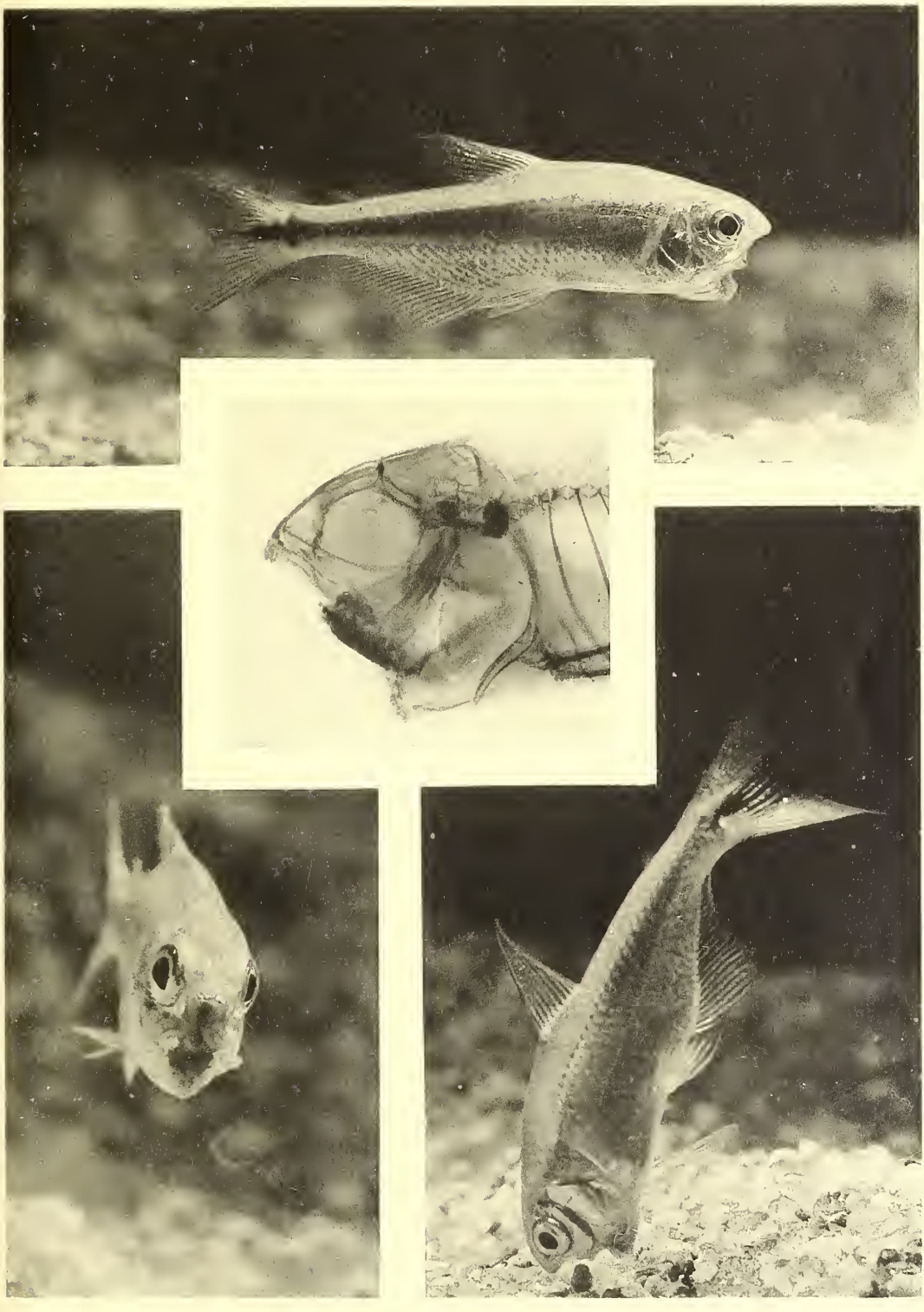
Astyanax mexicanus lacking a lower jaw.

Upper: Lateral view of the living fish in an aquarium.

Lower left: Frontal view of the above fish. The teeth in the upper jaw are clearly visible as is the slit-like "mouth."

Lower right: View of the fish about to pick up a particle of fish-food from the bottom, showing the position assumed by the fish at such times. The particle is directly below the mouth opening. (Photos by W. Chavin).

Middle: Radiograph of the head of the newly-preserved fish. (Radiograph by E. Logan).



A SECOND CASE OF SURVIVAL BY A TELEOST WITHOUT A LOWER JAW

3

The Nature of Post-larval Transformation in *Tylosurus acus* (Lacépède)

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(Plate I; Text-figures 1-9)

Introduction

THE transformation of the post-larvae of *Tylosurus raphidoma* (Ranzani) has been shown by Breder & Rasquin (1952) to include a process of sloughing off melanic areas of the dorsal fin. Since that report was published, data on comparable stages of development for the related and in many ways similar *Tylosurus acus* (Lacépède) have been obtained. There are, however, rather remarkable and striking differences, the demonstration of which forms the bulk of this communication.

Since the two species are sympatric, it has seemed strange that with their close superficial similarities they would apparently occupy the same environmental locus. The detailed data of this paper should improve understanding of the reasons for this situation. The comparisons it has been necessary to make in order to clarify differences and similarities should be useful in the taxonomic separation of these two forms at any stage of development.

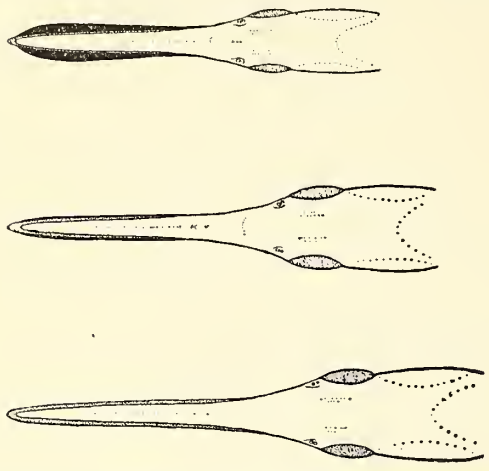
The studies and new material reported herein were made possible by the Lerner Marine Laboratory on Bimini, Bahamas.

Description of Transformation

The gross changes in any fish at the time of transformation from a post-larval individual to the adult form are obviously a function of growth in the sense of increase in size and the differential rates of various aspects of this growth, or heterogony. This is based in final analysis on the behavior of the cellular components of the tissues involved and properly belongs in the province of histology. For this reason this descriptive section is divided into two parts, the first dealing with the gross changes in the fish and the second with the accompanying histological changes.

Growth and Heterogony. — In the earliest stages known, the lower jaw of *Tylosurus acus* protrudes considerably beyond that of the upper in a manner not unlike that of many species of the related genus *Strongylura*. This is the so-called "halfbeak" condition of the young of these fishes in which they resemble the adult in the related Hemirhamphidae. The beak reduces rapidly as the growth rate of the upper jaw exceeds that of the lower so that at a length of about 250 mm standard length the two jaws are very nearly the same length. This condition they retain throughout life. At about the same time, i.e., when the adult form of the beak is attained, there is a transient development of mandibular lappets which quickly reduce themselves to a mere dark line on the jaw. The sizes at which this change takes place are shown in three stages in Text-fig. 1, from the maximum development to the loss of the appendages.

Concurrently appearing with the loss of this "halfbeak" condition is the development of an elevation of the posterior part of the dorsal fin, which becomes intensely black. This too reduces but does not disappear until some time later. The reduction of this area of the fin is shown in Text-fig. 2. Before the dark elevation of the posterior of the dorsal fin has reached its fullest development, the anterior rays begin to increase in length. On attaining a certain size the anterior rays retain that increased proportion for the remainder of the fish's life so that the fully adult fish has a dorsal fin high anteriorly and low posteriorly.

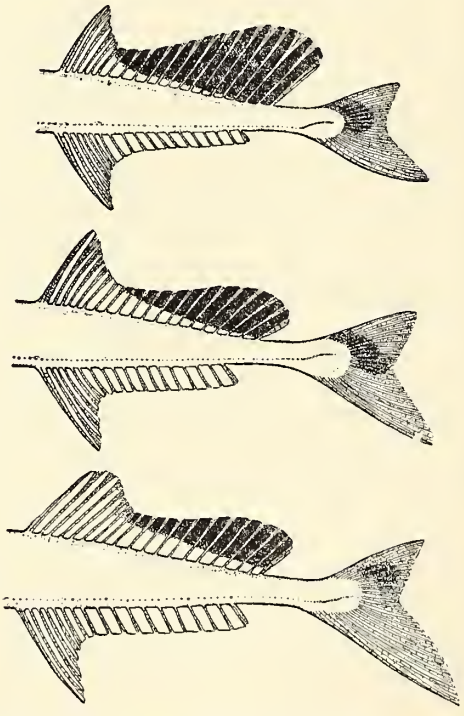


TEXT-FIG. 1. Heads of *Tylosurus acus,* showing the maximum development of the mandibular lappets and their subsequent reduction. Upper, 205 mm, s.l., middle, 205 mm s.l., lower, 242 mm s.l.

Numerical data supporting the above will be found in Tables I, II and III. The changes in the anterior and posterior heights of the dorsal fin are shown graphically in Text-fig. 3 with these values shown as percentages of the standard length of the fishes. Text-fig. 5 shows the same data expressed in millimeters. The interesting differences in these two sets of curves based on the same data are a measure of the heterogony responsible for these transformations. Text-fig. 7 shows graphically the changes in the head, beak and mandibular lappets. The comparison with *Tylosurus raphidoma* which these several graphs present is given consideration in the discussion.

Histology of Fin Transformation. — A post-larval fin was removed from a specimen fixed in Bouin's solution and an intermediate stage fin was removed from a formalin-fixed specimen. Both were sectioned at right angles to the long axis of the fin rays at seven microns and alternate slides were stained with Harris's hematoxylin and eosin and Masson's trichrome connective tissue stain. These are comparable to two fins of *T. raphidoma* studied by Breder & Rasquin (1952) and two photographs from that report are republished here for the purpose of comparison. Plate I, Figure 1, is a detail of the intact fin of *T. raphidoma* before the onset of disintegration and may be compared with Figure 2 which is a photomicrograph of a fin of *T. acus* before the onset of resorption. Figure 3 shows the inter-radial membrane of the disintegrating part of the dorsal fin of *T. raphidoma* and may be compared with Figure 4, which is a detail of the inter-radial membrane of the dorsal fin of *T. acus* during the period of resorption.

Fundamentally the structure of the dorsal fin of *T. acus* is the same as that of *T. raphidoma,* described by Breder & Rasquin (1952). Actinotrichia are present on the tips of the rays extending distally from between the most distal segments of the lepidotrichia. Nerves, blood vessels and connective tissues are arranged in the fin elements in the same way in both species. The area about the first ray of the fin is broader than around the rest of the rays and contains more connective tissue. Here the blood vessels are larger than capillaries, although still extremely thin-walled, and contain coarse granular eosinophiles. In fact these cells are more frequently seen than erythrocytes. At the level where the last division of the lepidotrichia of the first ray is seen, the coarse granular cells are found in the connective tissue spaces as well. In this area also the blood vessels appear filled with an acellular material that is coarser and more granular than serum usually appears in section. It seems possible that the acellular fluid found in these blood vessels is less dilute than that found in other parts of the body, owing to the accumulation of products of beginning dissolution of the fin tissues. Coarse granulocytes are common in the blood vessels at the



TEXT-FIG. 2. Tails of *Tylosurus acus,* showing the maximum development of the black posterior dorsal elevation and its subsequent reduction. These fish are the same as those of Text-fig. 1.

TABLE I. MEASUREMENTS (IN MILLIMETERS) OF THE DEVELOPMENT OF THE DORSAL FIN OF *Tylosurus acus*

Standard length	Posterior dorsal height	Anterior dorsal height	P.D.H./ S.L.	A.D.H./ S.L.
23	1.27	0.18	0.06—	0.01—
35	1.56	0.70	0.04+	0.02
79	2.10	2.97	0.03—	0.04—
205	17.00	15.00	0.08+	0.07+
215	11.50	16.50	0.05+	0.08—
242	11.50	17.00	0.05—	0.07+
620	12.00	36.00	0.02—	0.06+

Note. Fish in the 200 mm class from Bimini, others from the Dry Tortugas.

base of the fin where it meets the dorsal musculature. In both fins of *T. acus* these coarse granular eosinophiles are never at any place as numerous as they appeared in *T. raphidoma,* although they are more numerous in the resorbing fin than in the intact one.

Breder & Rasquin (1952) have suggested that the appearance of quantities of coarse granular eosinophiles is associated with a function of preserving fat from the degenerating tissue for the further physiological economy of the fish. These cells are also often found in abundance in parasitized teleosts. They were extremely abundant in the fins of *T. raphidoma* that also showed many encysted worms. The sections of *T. acus* studied for the present report showed no parasites, and while the coarse granulocytes were not so abundant as they were in the parasitized fish, they were still found in great numbers. Jordan & Speidel (1931) have suggested that they are responsible for the transportation of fat in the African lungfish. Their presence in degenerating fins of *Tylosurus acus* also seems to indicate that they have some function in the resorption of tissue unrelated to the presence of parasites.

The striking differences in histological structure between the dorsal fins of the two species lie in the structure of the epithelium and in the number of melanophores. In *T. acus,* the covering epithelium of the young intact fin may be from six to ten cell layers deep while in *T. raphidoma* it is extremely thin, being only one or two layers thick. Within the covering epithelium in the fin of *T. acus* the connective tissue shows two lines of melanophores and between them can be seen an occasional capillary and connective tissue fibers and cells. In *T. raphidoma* these details are obscured by the density of the melanic tissue (Plate I, Figures 1 and 2).

The resorbing fin of *T. acus* contrasts sharply with the disintegrating fin of *T. raphidoma.* Where the latter shows naked rays devoid of epithelium, and ragged, disintegrating inter-radial membranes, the resorbing fin of *T. acus* shows the epithelium to be intact over the entire surface of the fin. There appears to be some breakdown of the cellular boundaries of the melanophores. In many places, melanin granules are seen in the epithelium being passed through to the surface. These discrete granules are obviously not contained within melanophores. At the base of the resorbing fin the connective tissue is filled with coarse granular eosinophiles and macrophages. The phagocytic cells are filled with cellular debris and melanin granules. Many of the coarse granulocytes appear to have disintegrated, liberating their granules into the meshes of the loose connective tissue.

These three items—the loss of melanin through the epithelium of the fin and phagocytosis of melanin at the base of the fin, the abundance of coarse granulocytes, and the phagocytosis of cellular elements—are the only histological signs of resorption of tissue. The resorbing fin is somewhat more delicate in structure than that of the post-larval form in that the epithelium contains fewer layers of cells and the connective tissue of the inter-radial membrane is less dense and shows fewer nuclei. Unlike the disintegrating

TABLE II. DEVELOPMENT OF THE BEAKS OF *Tylosurus.* (BASED ONLY ON SPECIMENS WITH UNBROKEN BEAKS).

Standard length	Upper jaw[1]	Lower jaw[1]	Difference	Per cent difference
		Tylosurus acus		
23	1.2	3.0	1.8	60
28	3.0	6.5	3.5	54—
30	2.0	4.0	2.0	50
35	5.0	8.5	4.1	41
42	6.5	11.5	5.0	43
97	21.0	30.0	11.0	37—
205	38.5	41.0	2.5	6+
215	45.0	48.0	3.0	6+
242	52.0	53.5	1.5	3—
590	134.0	138.0	4.0	3—
660	130.0	134.0	4.0	3—
		Tylosurus raphidoma		
11	0.5	0.8	0.3	37+
19	1.9	2.1	0.2	10—
44	21.2	22.0	0.8	4—
149	28.2	29.8	1.6	5—
231	48.6	50.1	1.5	3—
500	106.0	109.5	3.5	3—

[1] Measured from eye. All in millimeters. From Breder (1932) with additions.

TABLE III. PROPORTIONS OF MANDIBULAR APPENDAGES COVERING ONLY THE PERIOD OF THEIR PRESENCE. (ALL MEASUREMENTS IN MILLIMETERS. BASED ONLY ON SPECIMENS WITH UNBROKEN BEAKS).

Standard length	Length of lower jaw	Mandibular appendages[1]		Percent of lower jaw	
		Greatest width	Distance of G. W. from jaw tip	Greatest width	Distance from tip
		Tylosurus acus			
97	30.0	—	—	—	—
205	41.0	6.0	24.0	15—	59—
215	48.0	4.0	24.0	8+	50
242	53.5	—	—	—	—
		Tylosurus raphidoma			
11	0.8	—	—	—	—
19	2.1	0.6—	0.4—	29—	19—
44	22.0	10.0	4.0	45+	18+
149	29.8	10.9+	7.0—	37—	23+
231	50.1	—	—	—	—

[1] The specimen nearest in size above and below those with mandibular appendages is given in each species.

dorsal fin of *T. raphidoma,* that of *T. acus* shows no breakdown of connective tissue elements, no sloughing off of epithelium, and no sign of weakening of the structure of the rays. The loss of tissue from this fin is not accomplished by a violent disruption of tissue structures.

DISCUSSION

Comparison with Tylosurus raphidoma.— Aside from the aberrant and much flattened *Ablennes hians* (Cuvier & Valenciennes), *Tylosurus acus* and *T. raphidoma* are the only members of the family Belonidae in the West Indies and adjacent waters which reach a length of several feet or more. The smaller species, usually going under the generic name *Strongylura,* differ from these two species in many respects, a number of which are not easily handled by conventional taxonomic methods. The characters usually used to separate these two quite different types of fishes are at best trivial. This has led to the suppression of *Tylosurus* by various recent authors. We have followed a more conservative usage in the retention of the name *Tylosurus* for these larger and more widely ranging forms with higher dorsal and anal fin formulae, pending more satisfactory analysis of the status and limits of genera in this family.

In ontogeny various characters in the West Indian species of the two groups show the following readily recognized differences.

a. Beak usually not more than 2 times rest of head in adults; dorsal and anal fins with 20 or more rays; juveniles pass through a stage with a high dark-colored prolongation of the posterior part of the dorsal and possess dark mandibular lappets; dark vertical bars are present under certain conditions of environment. *Tylosurus*

b. Beak usually more than 2 times rest of head in adults; dorsal and anal fins with less than 20 rays; juveniles pass through no such stage as described above, but the "half-beak" stage is much more marked, this being somewhat suppressed in *Tylosurus;* a narrow, longitudinal, dark or iridescent stripe on the sides usually present, but no vertical bars. *Strongylura*

The larger and more widely ranging West Indian species of *Tylosurus* are more given to open and deep water while the smaller *Strongylura* favor water shallower and more confined, such as about docks and in mangrove passages.

Although there is no real taxonomic difficulty in separating large specimens of the two species of *Tylosurus* under consideration, some of the developmental stages may present difficulty. The following data should be helpful in questionable cases and, together with certain of the included diagrams, should make clear separation possible in all instances.

ADULTS

a. Beak about 2 times rest of head (about 66% of head). Scales about 380-400.
Body depth 18.5 to 22 in standard length (about 4-5%) 6-7 in head, (about 12-16%).
Dorsal surface usually notably bluish.
Tends to stay outside of harbors.
Pupil about 2¾ in longest diameter of eye (about 36%).
Umbelacrum larger and accompanied with a patch of corneal pigment.

Angle of visual deflection about 13-140° below the horizontal[1]. *Tylosurus acus*

b. Beak about 1½-1⅝ times rest of head (about 57-60% of head).
Scales about 350.
Body depth 13.3-18 in standard length (about 5-7%), 4.35-5.8 in head, (about 17-23%).
Dorsal surface usually notably greenish.
Tends to stay inside harbors.
Pupil about 2⅓ in longest diameter of eye (about 42%).
Umbelacrum smaller and with corneal pigment reduced to a few flecks.
Angle of visual deflection about 9-10° below the horizontal[1].
Tylosurus raphidoma

JUVENILES

a. A definite "halfbeak" condition is passed through; see Text-fig. 7.
Labial folds slightly developed and not angulated; see Text-fig. 7.
Pectorals usually uniform hyaline or dusky.
Tylosurus acus

b. No pronounced "halfbeak" condition at any time, jaws nearly co-terminous at all sizes; see Text-fig. 7.
Labial folds well developed and angulate; see Text-fig. 7.
Pectoral tips usually blackish.
Tylosurus raphidoma

[1] The angle of visual deflection is most easily measured by taking from the fish the distance between the orbital rims at top (interorbital distance) and at bottom and the vertical diameter of the eye. Then with this data the angle which the optical axis (at rest) makes with the horizontal may be calculated according to the following formula. Let

a = distance between opposite orbital rims at top
b = distance between opposite orbital rims at bottom
c = vertical diameter of eye (measured along its face, not as a projection on a vertical plane)

These values define a symmetrical trapezoid with the lower base, b, smaller than the upper base, a, and the two non-parallel sides each equal to c. The altitude on this figure, which may be called d, forms a leg of a right triangle with hypotenuse c and the other leg $\frac{a-b}{2}$. A perpendicular from c to the opposite angle, the right angle of this triangle, forms a smaller and similar triangle with its hypotenuse $\frac{a-b}{2}$. Since $\frac{a-b}{2}$ is horizontal and the perpendicular to c is parallel to a perpendicular at its midpoint (the optical axis), the angle between $\frac{a-b}{2}$ and the perpendicular to c is the angle sought. Since this triangle is similar to the larger triangle on which it is based, this angle is equal to the angle between c and d which is opposite $\frac{a-b}{2}$ in the larger triangle. Calling this angle A, then

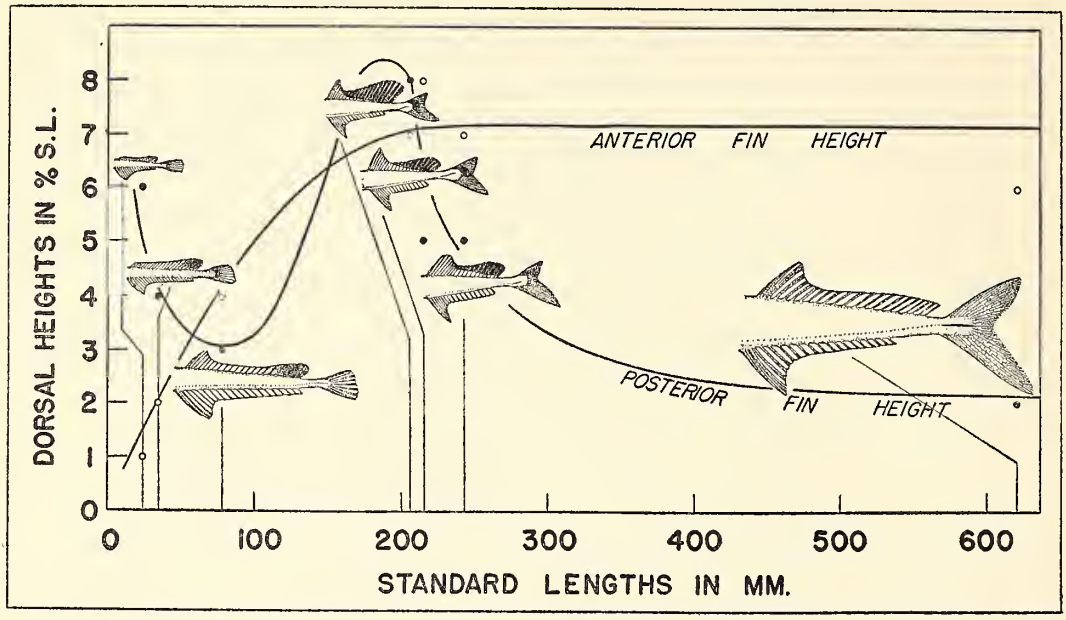
$$\sin A = \frac{a-b}{2c}.$$

The developmental differences and similarities between the two species of *Tylosurus* are so completely illustrated in Text-figs. 3-9, inclusive, that little explanation is necessary. The full development of the high dark posterior dorsal is attained at a slightly larger size in *T. acus*, about 200 mm as against about 150 mm in *T. raphidoma*. It is noteworthy, too, that at the very early stages it is relatively higher in respect to the length of the fish than a little later, which gives a depression in the curve as shown in Text-fig. 3. This deflection is absent in *T. raphidoma*, where it simply rises proportionally from the earliest stages, as may be seen in Text-fig. 4. The development of the anterior rays is evidently practically identical in both forms, as is evident from an examination of the two text-figures above noted. Apparent differences would seem to be due to the relative paucity of the rarer *T. acus* material.

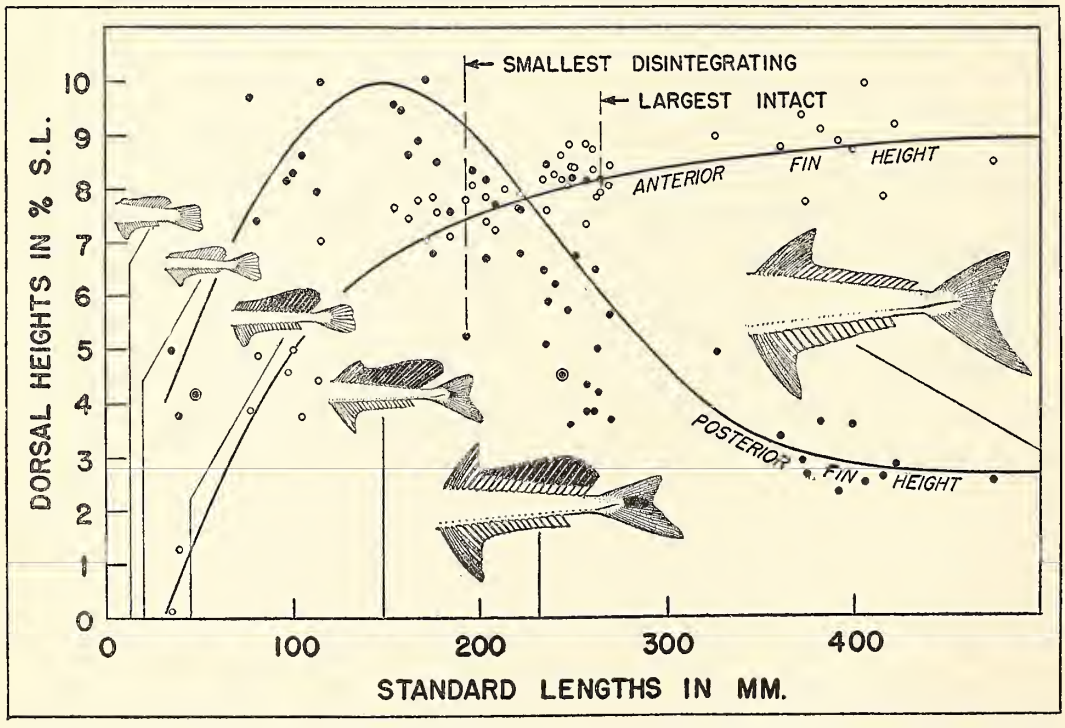
The same data plotted not in relation to the length of the fish but in reference to absolute measurements, emphasize these differences, as indicated in Text-figs. 5 and 6. The similarity of the growth of the anterior part of the dorsal fin in both species and the sloughing of the posterior lobe in *T. raphidoma* and its disappearance by resorption in *T. acus* is clearly indicated. Here it is seen that *T. acus* reaches the peak of its posterior lobe development at just about the place where the first sloughing occurs in the most precocious *T. raphidoma*.

The development of the beaks of both species is plotted comparatively in Text-fig. 7. By plotting the standard length against the percentage of difference of the excess of the lower jaw over the upper, from Table II, the ontogenetic differences between the two forms in respect to this feature are clearly developed. This diagram indicates how much farther the suppression of the "halfbeak" juvenile condition has progressed in *T. raphidoma* as compared with *T. acus*. It again shows the relative sizes at which the development of the mandibular lappets is at maximum and the illustrations point out how much greater these structures are developed in *T. raphidoma*, together with their different outlines.

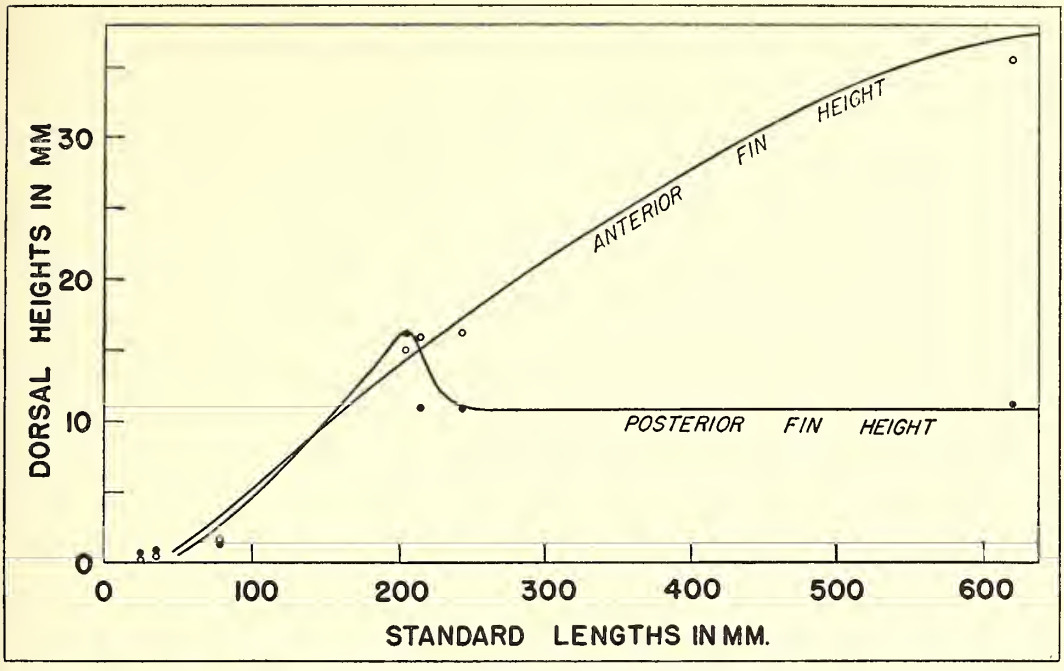
The growth and attainment of maturity would seem to be rather similar in these two species, insofar as we are able to interpret the somewhat limited material. Text-fig. 8 shows clearly that in *T. acus* young fish under 100 mm have been collected only in June and July while fish between 200 and 250 mm have been taken only in October and November. Various individuals that were seen about the laboratory dock in January and February, 1952, under conditions that made catching out of the question, were carefully estimated to be about 300 mm in standard length. The Florida fish of 150 mm



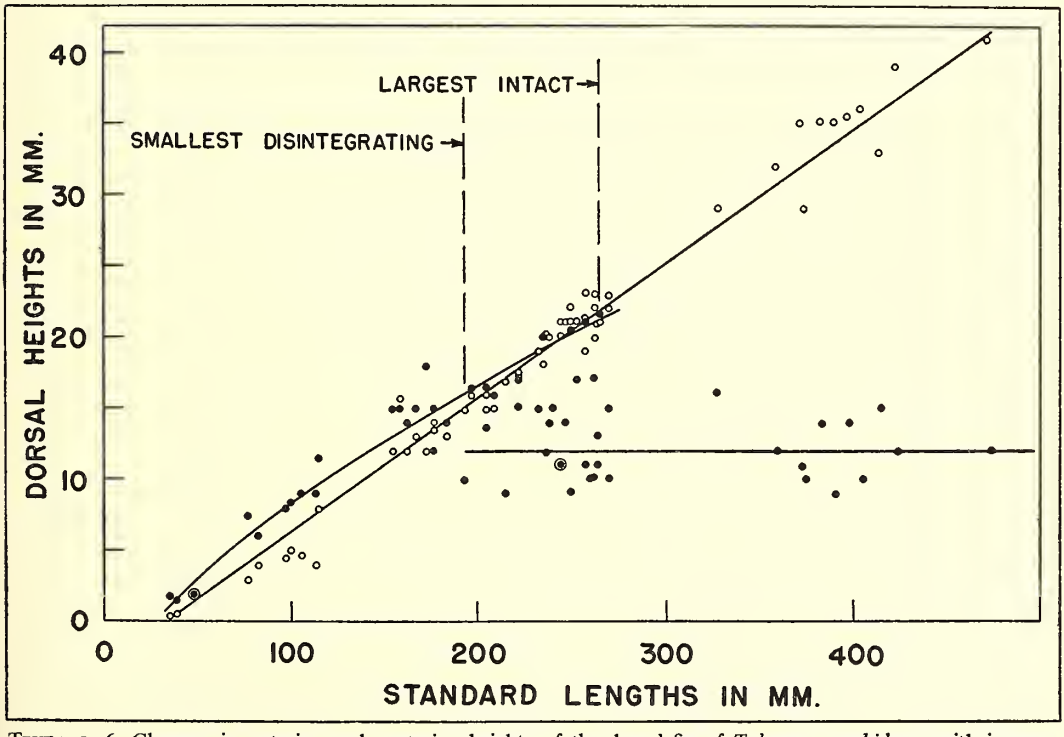
TEXT-FIG. 3. Changes in anterior and posterior heights of the dorsal fin of *Tylosurus acus* with increase in length of fish. Dorsal heights expressed as per cent of standard lengths. White circles represent anterior fin height. Black circles represent posterior fin height. Curves are freehand approximations of the mean. The illustration of the three smallest and the largest stages have been taken from Breder (1932). These with the new material show the appearance of the fish at the indicated lengths. Data from Table I.



TEXT-FIG. 4. Changes in anterior and posterior heights of the dorsal fin of *Tylosurus raphidoma* with increase of length of fish. Dorsal heights expressed as per cent of standard lengths. White circles represent anterior fin height. Black circles represent posterior fin height. Curves are freehand approximations of the mean. No account is taken here of the mechanics of the changes in the posterior height. The illustrations of various indicated stages at their respective lengths have been taken from Breder (1932). Text-figure from Breder & Rasquin (1952) for direct comparison of these two sympatric species.



TEXT-FIG. 5. Changes in the anterior and posterior heights of the dorsal fin of *Tylosurus acus* with increase in length of fish. Unlike Text-fig. 3, to which this should be considered complementary, the dorsal heights are expressed not in relation to the length but in absolute units. White circles represent anterior fin height. Black circles represent posterior fin height. Curves are freehand approximations of the mean. Data from Table I.



TEXT-FIG. 6. Changes in anterior and posterior heights of the dorsal fin of *Tylosurus raphidoma* with increase of length of fish. Unlike Text-fig. 4, to which this should be considered complementary, the dorsal heights are expressed not in relation to the length but in absolute units. White circles represent anterior fin height. Black circles represent posterior fin height. Curves are freehand approximations of the mean. Account is taken of mechanics of the posterior height indicating its disruptive nature. From Breder & Rasquin (1952) for direct comparison of these two sympatric species.

in April may have been a late-spawned fish and somewhat retarded, as is suggested on the graph, or may be a very precocious individual of the first year. Partly spent females were seen at the Dry Tortugas in July, 1929. This agrees well with the sizes of the small fish and suggests that sexual maturity is attained at about the same size and age as in *T. raphidoma*. The large fish listed in Table IV, over 1,000 mm long, is not included in Text-fig. 8, as it could not be placed with any degree of reasonable judgment. It would seem most likely to be in its 4th or 5th year, but could be considerably older if the life span of these fishes permits such ages. More material than is at present available would be necessary to warrant further analysis along this line.

Although these are considered sympatric species and in fact both species have been caught in a single setting of a gill net, the fact remains that they show differential response to the finer details of their environment. *T. acus* is much less likely to be found in the harbor at Bimini than is *T. raphidoma,* which is regularly present, including large sized individuals that may frequently be seen chasing *Hemirhamphus* and other small prey. No *T. acus* were taken or seen in the harbor until a considerable amount of dredging had materially changed the depths about the dock. Almost immediately the dock-side fauna altered in the direction of more oceanic species. These included young *T. acus* as well as small *Cypselurus, Coryphaena, Histrio,* etc.

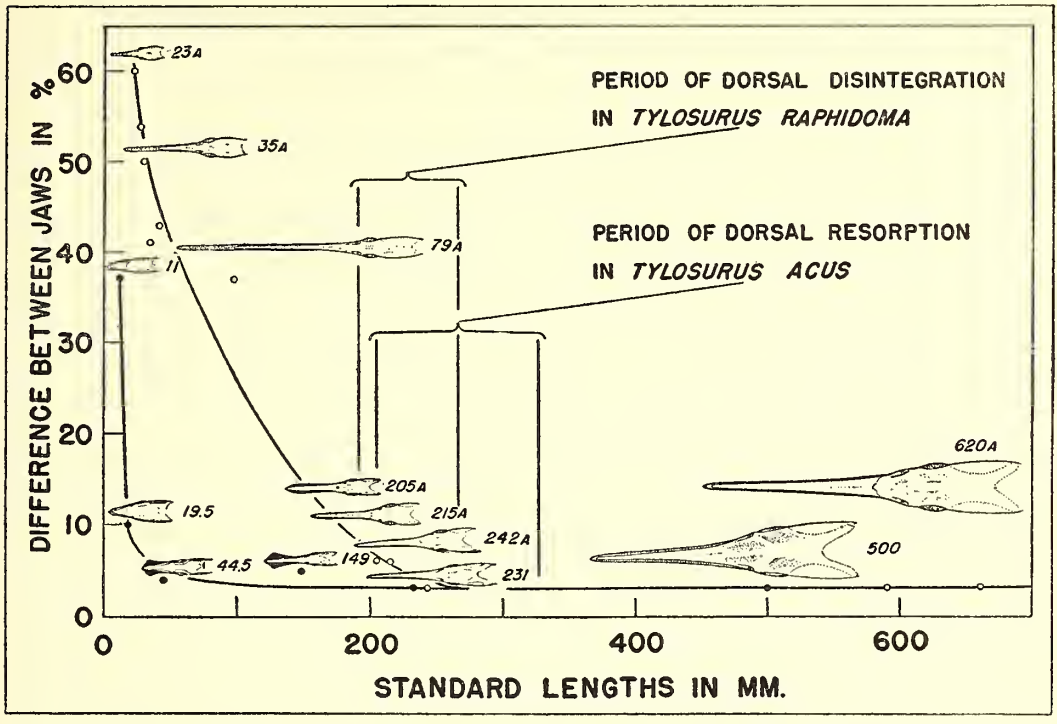
The large *T. acus* caught outside the harbor by angling are normally a brilliant ocean blue on the back, a color common to many truly oceanic fishes. The young ones taken about the dock showed the blue color also but they had evidently just entered the harbor. On the other hand, large *T. raphidoma,* taken inside the harbor, near its mouth or over shoal water, have been noted in each instance to be a bright green. Both these colors are evidently matching the two different environments, and it may be that this is strictly a pigmentary response and that both species are capable of showing both colors. If this is so, then this noted difference surely reflects environmental choice, which is evidently different in the two forms. The large umbelacrum, greater amount of corneal pigment and smaller pupil in *T. acus* may be a reflection of this also, as this fish is fully exposed at the surface of the sea in open water to a greater extent than *T. raphidoma.* The depression of the visual angle, which is more marked in the former species, may be associated with danger from more nearly directly below than that from which *T. raphidoma* would be likely to suffer in its more shallow environs.

Both species are voracious and will strike at nearly any fish or other organism small enough to be managed. Two *T. acus* of about 200 mm were maintained in a 12-foot circular pool for eight days with a large school of *Jenkinsia.* The latter performed with regard to temperature as described in detail by Breder (1951); that is, they would come to a thermal barrier of a fraction of a degree and refuse to go into the differently temperatured water, regardless of the presence or driving influence of man or the small *Tylosurus.* These latter had no such regard for the finer differences of temperature and ranged at will about the pool, picking off *Jen-*

Table IV. Dates of Collection and Standard Lengths (in Millimeters) of *Tylosurus acus.*

Locality	Date	No. of fishes	Min.	Modes[1] or Means	Max.
Nassau, Bahamas[2]	2/11/30	1	—	620	—
Dry Tortugas[2]	6/4/29	1	—	97	—
	6/13/29	14	16	*22*	42
	7/1/29	1	—	38	—
	7/6/29	1	—	660	—
Sandy Hook, N. J.[2]	7/19/28	1	—	805	—
Key West, Fla.[2]	6/—/18	10	30	*35*	46
Biscayne Bay, Fla.[3]	4/20/17	1	—	circa 152	—
Woods Hole, Mass.[3]	7/27/86	1	—	circa 1,219	—
Bimini, Bahamas	10/25/51	3	205	221	242
	11/2/51	1	—	203	—
	11/10/51	3	194	209	232
	11/2/49	1	—	190	—
	1-2/—/52	10+	—	est. 300+	—

[1] Where possible, modes are used in preference to means; the former are in italics.
[2] From Breder (1932).
[3] From Nichols & Breder (1926).

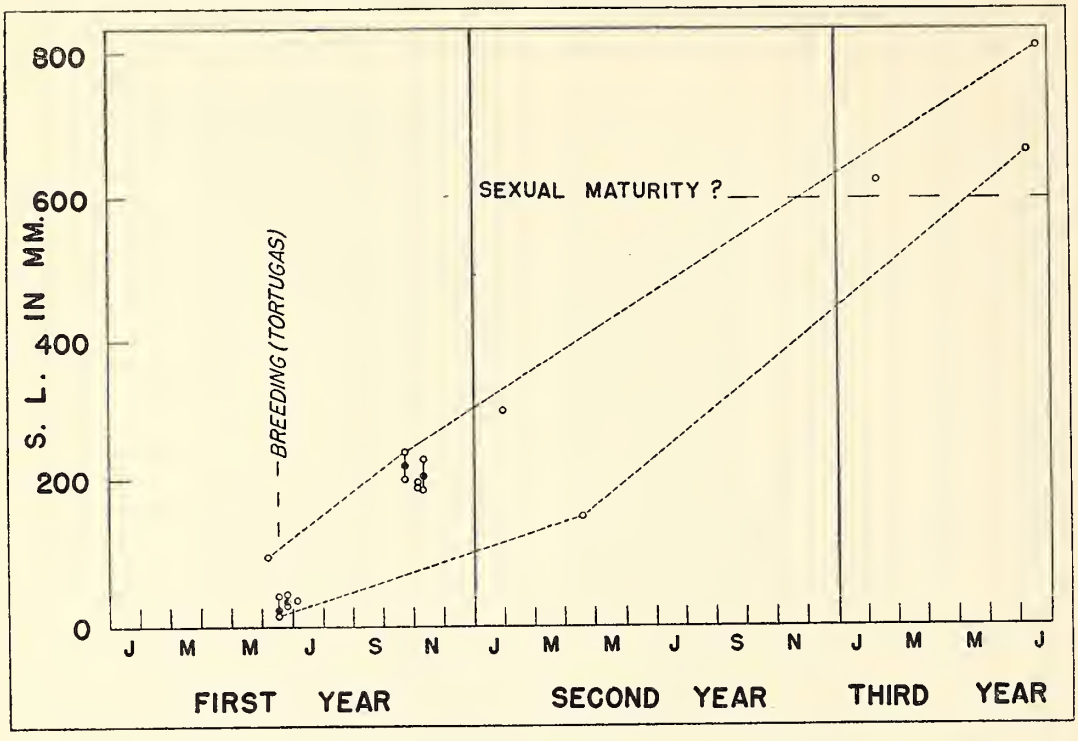


Text-fig. 7. Changes in the relative lengths of the upper and lower jaw in *Tylosurus acus* and *T. raphidoma*. The illustration of the various indicated stages of the beak and its mandibular appendages are taken from Breder (1932) in the case of the latter species, as well as the former except for additional material presented herewith. The numbers near the fish heads indicate standard length of fish in mm. An "A" after such numbers indicates *T. acus*, while lack of a letter following the number indicates *T. raphidoma*.

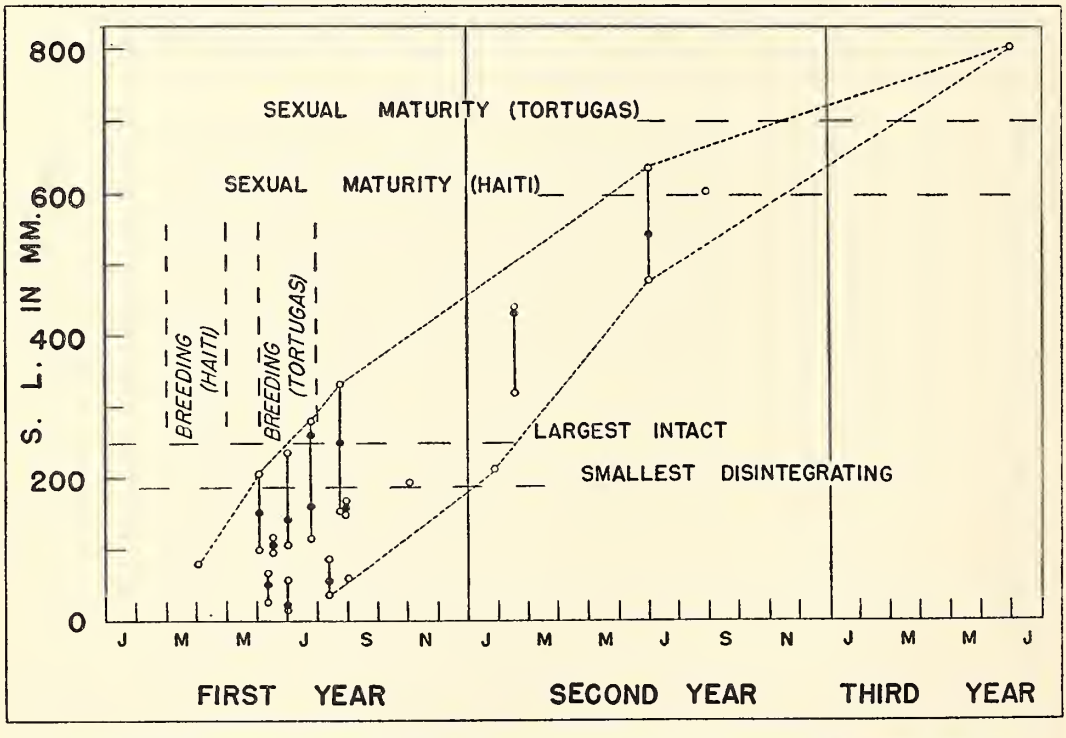
kinsia at short intervals. This caused no great commotion in the school of these fish, only a slight "shock wave" passing through it, as when a pebble is dropped. The presence of the predatory *Tylosurus* as such seemed to have no special significance for the herrings; they were evidently regarded merely as any other solid object to be avoided, provided such behavior did not violate the fine regard these fishes have for both temperature and light variations.

The regular feeding of the *Tylosurus* pointed up their need for a practically continual food supply, evidently required because of the incredibly short synentognath gut—a straight tube from gullet to vent.

Phylogenetic Significance.—The phylogenetic significance of the ontogenetic differences displayed by these two species is not easily established. If we take the conventionally accepted general phylogeny of the synentognaths as discussed by Schlesinger (1909), Regan (1911) Sewertzoff (1927) and Nichols & Breder (1928) and reinforced by de Beer (1951), *T. acus* could be placed as more primitive than *T. raphidoma* on the basis of several features. The Hemirhamphidae, which as adults resemble the young of most of the Belonidae because of the retention of the "halfbeaked" condition, can be considered practically as shortened neotenous belonids. Since this feature is reduced in what we here have called *Tylosurus* as compared with *Strongylura,* the latter could be considered more primitive. Within the *Tylosurus* group *T. raphidoma* has practically suppressed this juvenile character, far more so than has *T. acus.* With this has gone a change from a more slender condition, which is reflected in the fact that *T. acus* as compared with *T. raphidoma* is a slimmer fish, with a longer beak, less body depth and a higher scale count. The development of the mandibular lappets is in keeping with this general change in that they are narrower in the former and evidently do not last as long as they do in *T. raphidoma.* This feature might well be the manner in which such a new character puts in its appearance. The fact that there are "erratic" presences and absences of barbels throughout the order may only be a case of the



TEXT-FIG. 8. Standard lengths of *Tylosurus acus* and their respective dates of occurrence.



TEXT-FIG. 9. Standard lengths of 279 *Tylosurus raphidoma* and their respective dates of occurrence. From Breder & Rasquin (1952) for direct comparison of these two sympatric species.

suppression or expression of the genetic potentialities of the group.

The whole problem in this group, as in so many others, is clouded by the fact that the phylogenetic "tree" may be inverted. A good many years ago Dr. George S. Myers discussed verbally the possibility of the non-exocoetid synentognaths being derived from or near the exocoetids rather than the reverse, as has generally been accepted. Breder (1938) discussed some of these inferences insofar as they concern the Exocoetidae. A very good case can be made out for the inversion. The most cogent reason against it, however, is given by de Beer (1951) who agreed with the earlier taxonomic workers in considering that the halfbeak features are the results of neoteny. Sewertzoff (1927) considered the very early stages of *Belone,* before either of the jaws began to lengthen, as passing through a stage equivalent to that of the short and equal-jawed flying fishes. It is believed, however, that this is a spurious resemblance since practically *any* fish embryo at the stages studied by Sewertzoff have short equal jaws; the point is that all fish jaws start off from such an early condition irrespective of what strange development subsequently may take place.

If the other synentognaths can be derived from the exocoetids, then the lengthening jaws, first the lower one in the hemirhamphids and finally both in the belonids, would have to be considered, in the usage of de Beer, as a hypermorphosis. If this were the case the series would be moving from short-bodied large-scaled forms to long-bodied fine-scaled forms. Whichever direction evolution moved, it is obvious that the transformations through which these fishes have gone is well in accord with the principles of distorted grids as discussed by Thompson (1942).

It should be evident that the determination of the direction of evolution in this group is of great importance in the interpretation of problems arising from the differences in the ways these two closely related fishes eliminate the juvenile high dark posterior lobe of the dorsal fin. So far as known the function of this appendage in *T. acus* would be similar to that supposed for *T. raphidoma* and discussed at length by Breder & Rasquin (1952). Since there are no data on the young of the former other than that contained in this paper, it would be pointless to speculate on whether the young of *T. acus* behaves in a manner similar to that of the young of *T. raphidoma* or whether this feature is employed in some other fashion.

However, it may be of some profit to speculate on which method of elimination of the dark dorsal lobe is antecedent. If we consider the condition found in *T. raphidoma* as the earlier, based perhaps on an organism's successful attempt to arrest an invasive melanosis, as has been suggested by Breder (in press), this would be then the first step in the reintegration of the organism's regain of control. The next step would be finally to substitute the ordinary process of resorption as seen in *T. acus* for the more violent and unusual one of sloughing off an intensely melanic area. That in both species the mandibular lappets are simply resorbed would suggest that only the large area involved in the dorsal fin made necessary an initial elimination by sloughing. Such would seem to be the case if the phylogenetic tree were inverted as above suggested. If, however, the more conventional position be accepted, the reverse would follow. Under such a supposition, the then more primitive *T. acus* with its moderately developed dorsal lobe transformed by the standard method of resorbing juvenile features. On the other hand, in *T. raphidoma,* in which this feature is actually more marked, as may be seen by reference to the several text-figures, it became no longer possible to resorb this material and recourse was made to the process of sloughing it off. If this is in truth the case, then the latter form may be "the end of the line." That is, if the sloughing process is no longer sufficient to preserve the integrity of the organism the next stage could conceivably be invasion and death. Actually, only one questionable case of fish melanoma has been reported to show metastases, Schlumberger & Lucké (1948) discussing Takahashi (1929). In the melanomas of the xiphophorin fishes extensively studied by Gordon (e.g., 1948 and 1951) death follows from invasive process only. Presumably in *Tylosurus,* if the area of melanosis were not sloughed off but went on to develop as a melanoma, the speed would be sufficient to preclude reproduction.

Such thought cannot help but make one wonder how many lines may have been eliminated in an evolutionary sense by some similar process. It would be perhaps inevitable in the in-and-out-breeding of organisms that this would follow. It would seem likely that more than occasionally an old line would reach some point where the gene combinations were such that it was impossible for the individuals to maintain organic integrity long enough to perpetuate their kind in adequate abundance.

It should be noted in conclusion that the raw data are adequate only to form a bare sketch of the ontogeny of *Tylosurus acus.* In the area available for collecting and study, *Tylosurus raphidoma* is much more abundant, as is evident from a comparison of the tabular data herein and that of Breder & Rasquin (1952) and Breder

(1932). It is also clear from the tables on both species that data during the height of the hurricane season, September and October, are virtually non-existent. Also much to be desired is further material of the larger sizes in sufficient quantity to make frequency studies. Only the availability of much more material suitably spread over the year can reduce the life history aspect of these species to a more refined and definitive form.

Summary

1. The loss of the high, dark posterior lobe of the dorsal fin is accomplished by a process of resorption in *Tylosurus acus* in contradistinction to *T. raphidoma* in which the loss of a homologous structure is accomplished by sloughing off the area.
2. Histologically, the post-larval dorsal fin of *T. acus* differs from that of *T. raphidoma* in that the epithelium covering the surface is six to ten cell layers thick, contrasted with the extremely thin epithelium of *T. raphidoma* of only one or two cell layers.
3. Melanophores are so few in the dark fin area of *T. acus* that connective tissue elements of the inter-radial membrane are readily visible, whereas in the same locus of *T. raphidoma* such details are completely obscured by melanin.
4. These two sympatric species occupy contiguous and overlapping ranges but are adjusted to different ecological factors, *T. acus* favoring a more offshore and *T. raphidoma* a more inshore environment.
5. The above-noted differences are reflected in a variety of structural, developmental and behavioristic details.
6. Because of the scant data on which the phylogeny of the group rests it is impossible at this time to evaluate clearly the difference between the methods of eliminating the post-larval dark posterior dorsal fin lobe.
7. It is tempting, however, to think of the dark area as an invasive area of melanosis which has been arrested in *T. raphidoma,* resulting in a sloughing off, and finally in *T. acus* as an integrated part of the life history in which the material is taken care of by the less violent process of resorption.

Bibliography

Breder, C. M., Jr.

1932. On the habits and development of certain Atlantic Synentognathi. Carnegie Inst. Washington Publ., 435: Papers Tortugas Lab., 28, 1:1-35.

1938. A contribution to the life histories of Atlantic Ocean flying fishes. Bull. Bingham Ocean. Coll., 6:1-126.

1951. Studies on the structure of the fish school. Bull. Amer. Mus. Nat. Hist., 98:1-28.

1952. The problem of directives to cellular proliferation as illustrated by ontogenetic processes in certain fishes. Growth, 16: 189-198.

Breder, C. M., Jr., & Priscilla Rasquin

1952. The sloughing of the melanic area of the dorsal fin, an ontogenetic process in *Tylosurus raphidoma.* Bull. Amer. Mus. Nat. Hist., 99:1-24.

de Beer, G. R.

1951. Embryos and ancestors. Revised ed. Oxford University Press, pp. 1-159.

Gordon, M.

1948. Effect of five pigment genes on the site of melanomas in fishes and the influence of two color genes on their pigmentation. In "The Biology of Melanomas," Spec. Publ., New York Acad. Sci., 4:216-268.

1951. Genetic and correlated studies of normal and atypical pigment cell growth. Growth, 10:153-219.

Jordan, H. E., & C. C. Speidel

1931. Blood formation in the African lungfish under normal conditions and under conditions of prolonged estivation and recovery. Jour. Morph. and Physiol., 51:319-371.

Nichols, J. T., & C. M. Breder, Jr.

1928. An annotated list of the Synentognathi with remarks on their development and relationships. Zoologica, 8:423-444.

Regan, C. T.

1911. The classification of the teleostean fishes of the order Synentognathi. Ann. Mag. Nat. Hist., 8 ser., 7:327-335.

Schlumberger, H. G., & B. Lucké

1948. Tumors of fishes, amphibians and reptiles. Cancer Research, vol. 8, 12:657-754.

Schlesinger, G.

1909. Zur Phylogenie und Ethologie der Scombresociden. Ver. Zool.-Bot. Ges., Wien, 59:302-339.

Sewertzoff, A. N.

1927. Uber die Beziehungen zwischen der Ontogenese und der Phylogenese der Tiere. Jenaische Zeitschr. fur Naturwissenschaft, 63:51-180.

Takahashi, K.
1929. Studie über die Fischgeschwülste. Zeitschr. Krebsforsch. 29:1-73.

Thompson, D. W.
1942. On growth and form. New ed. Cambridge Univ. Press, pp. 1-1116.

EXPLANATION OF THE PLATE

Plate I

Fig. 1. Detail of the intact dorsal fin of *T. raphidoma* before the onset of disintegration, showing thin epithelium and intense concentration of melanin. Magnification 450×. After Breder & Rasquin (1952).

Fig. 2. Detail of the intact dorsal fin of *T. acus* before the onset of resorption, showing thick epithelium and connective tissue elements and melanophores. Magnification 450×.

Fig. 3. Detail of the inter-radial membrane of the distintegrating dorsal fin of *T. raphidoma,* showing the loss of the epithelial layer and disintegration of connective tissue and melanophores. Magnification 450×. After Breder & Rasquin (1952).

Fig. 4. Detail of the inter-radial membrane of the resorbing dorsal fin of *T. acus,* showing no disintegration. Magnification 450×.

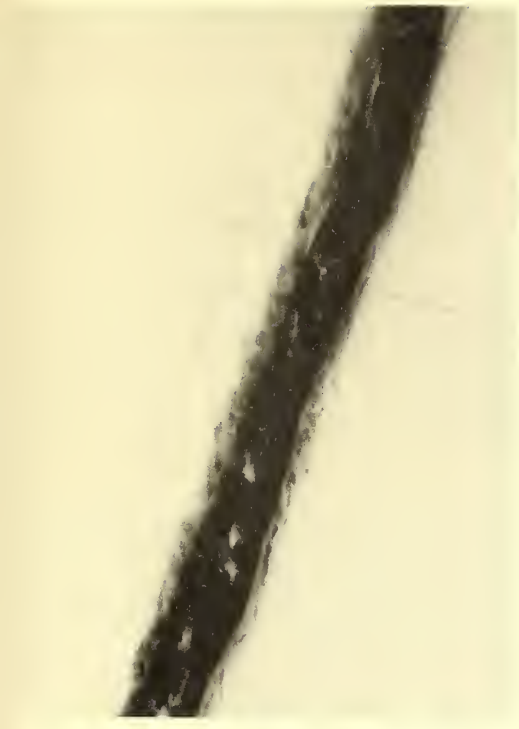


FIG. 1

FIG. 2

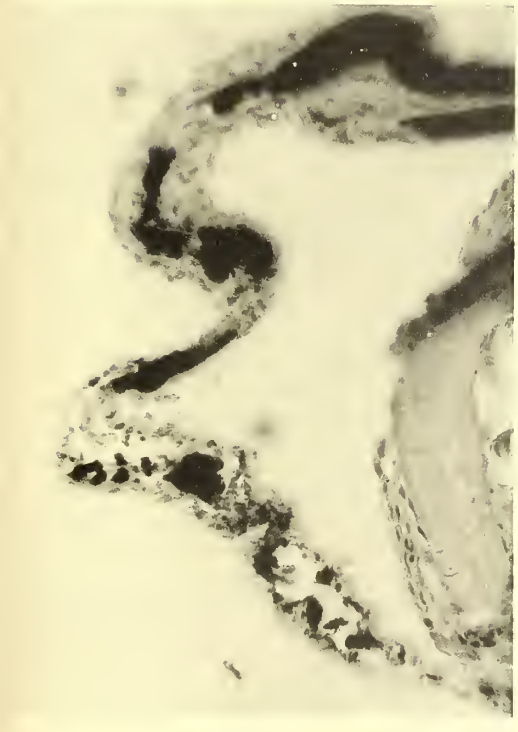


FIG. 3

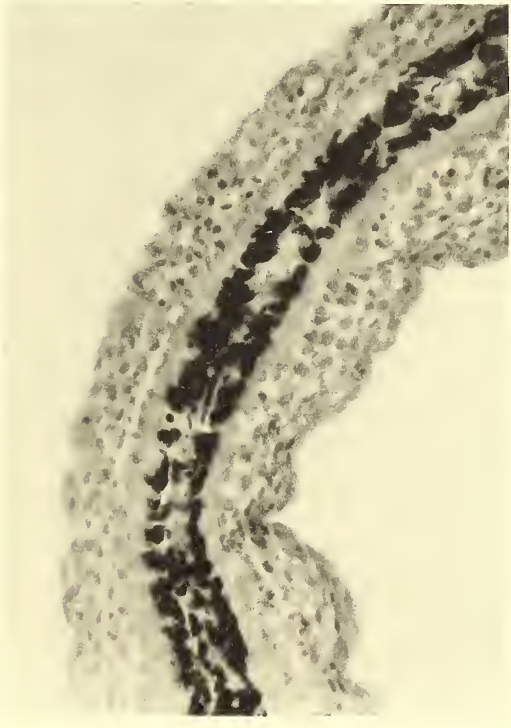


FIG. 4

THE NATURE OF POST-LARVAL TRANSFORMATION
IN TYLOSURUS ACUS (LACÉPÈDE)

4

Certain Measures of Intergradation and Divergence

Isaac Ginsburg

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(Text-figure 1)

TAXONOMISTS during the pioneering stages of the science generally established and described their species on the basis of one or very few specimens. To the present day some taxonomists pursue their studies according to the methods of the pioneers. Of necessity, all of us often have to follow the same course, because of the lack of adequate material. However, the information gained by a taxonomic study of one or a few specimens of a species gives a mere smattering of our knowledge of the species. It tells us very little or nothing about variability within the species, something we need to know in order to understand properly its morphology and its affinities to the species that are immediately related to it. Therefore, by and large, the trend in modern taxonomy, and rightly so, is toward extensive studies of intraspecific or intrapopulation variability, that is, the extent and manner of individual variability within the limits of the species, or population of lower rank, with regard to those characters in which natural populations diverge.

For an adequate knowledge of the relationship and differences between closely related species of the same genus, it is desirable and necessary to know the limits and manner of individual variability of their distinguishing characters. The same holds for the minor natural populations within the species. The differences or divergences between any one pair of closely related populations, for any given character, are ascertained by determining for each individual specimen within the sample studied the numerical value of the character studied. The individual data are then tabulated, and the difference between the two populations determined by comparing the evidence presented by the two sets of tabulated data.

As studies of intrapopulation variability increase in scope and extent, it becomes evident with ever-increasing forcefulness that the gap which many taxonomists, especially those of the past, thought exists between closely related species of the same genus, is often becoming ever more narrowed or obliterated altogether. The discovery is being made repeatedly that populations which have been, and should be, afforded full species status intergrade somewhat in the very characters that have been and are being used to distinguish them. Moreover, it is being discovered that divergences between pairs of closely related populations are of all degrees of magnitude and that no sharp or definite line can be drawn between the species category and that of subspecies.

Need for These Measures.—As divergences between closely related populations are of differing extents or degrees of magnitude, it becomes imperative to have some numerical measure of the degree of divergence between a pair of populations compared for a given character. This gives the taxonomist a criterion for drawing conclusions regarding the taxonomic rank to assign to the two populations which he studies and compares, namely, whether to designate them as full species or to a particular one of the several graded intraspecific categories in the hierarchy of lesser populations. In theory, the species may be conceived as the end point in, or as comprising, a graded series of lesser populations the relationship of which increases in relative remoteness, somewhat in the following order. The most intimately knit population, consisting of a group of individuals that are the offspring of the same parents, may be designated as the family (with a small "f"). Two or more closely related families may be said to constitute a clan; two or more related clans may be said to make up a tribe. Closely related tribes

theoretically constitute a variety; related varieties constitute a race; related races make a subspecies. In practice, the lesser of these minor categories are virtually altogether in the realm of theory at this stage of the science. At present we need consider only three of the upper intraspecific categories, the subspecies, the race, and the variety. In actual practice, in the great majority of instances, only the subspecies is taken cognizance of by most taxonomists. Perhaps what should more properly be designated race or variety is sometimes called subspecies. Some taxonomists use the terms race and subspecies synonymously to cover the concept of the same category.

For the present it would seem desirable to bestow a formal Latin name on the subspecies only and designate the race and variety by some informal designation, preferably one that indicates the geographic region that it inhabits, such as the "Chesapeake Bay race" or the "Potomac River variety." Perhaps in fewer instances the informal name might refer to the ecological niche that it occupies. That is, it seems best to refrain from going beyond the trinomial in nomenclature.

From the viewpoint of practicality, it is desirable that any such proposed measure have certain attributes. The taxonomist is a busy man and has to deal with thousands of populations of species rank or lower. It is, therefore, important for any such measure to be quickly determinable from the tabulated data. Also, the applicability of the derived number to the problem at hand and its pertinence in drawing acceptable taxonomic conclusions must be readily comprehensible. A proposed measure may have elegance and ingenuity from the mathematician's viewpoint, but if its derivation is too time-consuming and its pertinence not plainly evident, it will not serve as a useful tool to the taxonomist.

Some time ago I proposed two measures that seem to have the desired attributes (1938). One I called the index of intergradation, the other the index of divergence. These two indices are complementary; the greater the intergradation the less the divergence, and vice versa. For any given character compared between two populations the sum of the two indices equals 100. Some supplementary phases connected with this problem were considered by me in other papers (1937, 1939 and 1940).

Judged by private conversation with taxonomists, it seems that some misapprehensions exist in regard to the two indices as I proposed them, and the following statements seem to be in order. (1) My index of divergence cannot properly be described as the percentage of identifiable specimens in the two samples compared. (2) My index of intergradation is not represented by the percentage of the actual number of intergrading specimens in the two samples.

The paper cited above, by the use of actual taxonomic data, shows how the two proposed indices are applied in practice. The present paper discusses an underlying basis for these two indices. This may be done by considering two hypothetical populations.

Procedure.—Let us assume two natural populations, alpha and beta, that differ in a number of characters the most divergent one of which, or the principal character of which, is represented by the frequency distribution given in Table I. The class numbers might represent any character, such as the number of fin rays in a fish, the number of rays in a flower, the number of segments in the antenna of an insect, the tail length in a mouse or the length of the wing in a bird, in any chosen unit.

Table I.

Frequency Distribution of the Numerical Values of the Principal Character in Two Hypothetical Diverging Natural Populations, Alpha and Beta. (The function of the heavy vertical line, the dividing line, is discussed in the text).

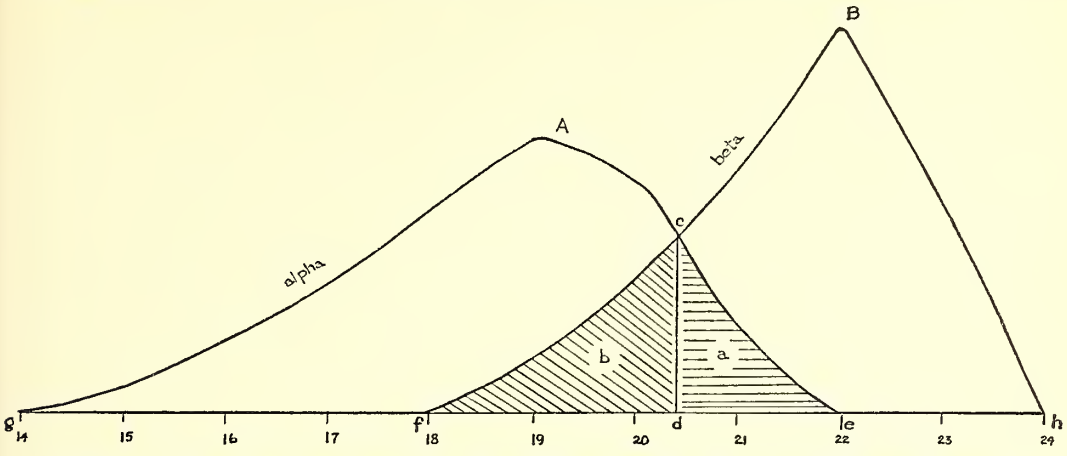
	Distribution								
Population	15	16	17	18	19	20	21	22	23
alpha	6	21	34	58	77	68	26		
beta					33	78	143	231	127

Changing the numerical frequencies in Table I to percentages of the total number of specimens in the two respective hypothetical samples, we get a distribution as given in Table II.

Table II.

Frequency Distribution of the Principal Character in Two Hypothetical Diverging Populations, Alpha and Beta, Based on the Same Assumed Data as in Table I, Except that the Frequencies Are Expressed as Percentages of the Two Respective Hypothetical Samples.

	Distribution								
Population	15	16	17	18	19	20	21	22	23
alpha	2.1	7.2	11.7	20.0	26.6	23.4	9.0		
beta					5.4	12.7	23.4	37.7	20.8



TEXT-FIG. 1.—Diagrammatic representation of the assumed data presented in Table II of two hypothetical populations, alpha and beta. Drawn by Mildred H. Carrington.

On the basis of the percentage figures in Table II, construct two overlapping curves (or polygons or histograms), as in Text-fig. 1, the curve on the left representing alpha, that on the right beta.

It should be stated at this point that the method here discussed is generally applicable to data derived from fairly homogeneous populations and based on fairly adequate samples. The comparison of two such sets of data from two closely related populations will usually result in two curves that intersect at one point. This will apply to the great majority of instances that may be encountered in taxonomic practice. In the relatively few special instances that may be expected, in which the distributions are irregular, and the curves intersect in more than one point, some modification of this method might be necessary.

Let the area enclosed by the curve and base line representing alpha be denoted by A and that representing beta by B.

From the point of intersection of the two curves draw a vertical line *cd* to the base line.

Then, the overlapped part of the graph may be considered as consisting of two—usually unequal—parts, namely, *ced* and *cfd* that may be said to represent the parts that A and B, respectively, contribute to the overlapped area.

Let areas *ced* and *cfd* be represented by *a* and *b*, respectively, *a* being longitudinally hatched in the graph and *b* obliquely hatched.

Index of Intergradation.—Now, let a′ represent the percentage of *a* into A, and b′ represent the percentage of *b* into B. Then,

$$(1)\quad a' = \frac{100\,a}{A}, \text{ and}$$

$$(2)\quad b' = \frac{100\,b}{B}$$

Combining equations (1) and (2) we have,

$$(3)\quad a' + b' = \frac{100\,a}{A} + \frac{100\,b}{B}$$

But B =A, since both curves are constructed on a percentage basis. Therefore,

$$(4)\quad a' + b' = \frac{100\,(a+b)}{A}$$

Dividing both sides of equation (4) by 2, we have

$$(5)\quad \frac{a' + b'}{2} = \frac{100\,(a+b)}{2A}$$

But $\frac{a' + b'}{2}$ equals my index of intergradation by definition. This means that this index of intergradation, when graphically presented, equals the percentage of the area overlapped by the two curves into the sum of the areas of both curves constructed on a percentage basis.

Index of Divergence.—Regarding my index of divergence, by definition it equals 100 minus the index of intergradation, that is,

$$\text{Index of divergence} = 100 - \frac{100\,(a+b)}{2A}$$

$$= \frac{100\,[2A - (a+b)]}{2A}$$

This means that, graphically presented, the index of divergence equals the percentage of the combined areas of the two curves diminished by the overlapped area, into the combined areas of the two curves. In other words, by reference to Text-fig. 1, it equals the percentage of areas *cgd* + *chd* into A + B.

Practical Use of Indices.—I have used these two indices with satisfactory results in drawing taxonomic conclusions in the following papers cited below: 1944: 376; 1950: 504 and 518; 1951: 198 (on last page cited "index of diver-

gence" erroneously printed for "index of intergradation"); 1952: 94; 1953: 35. In addition to the pages cited where the indices are formally applied, I applied them tacitly in drawing taxonomic conclusions in other places, from the determined data. In general, I found the idea of the use of these indices and their practical application of much help in the pursuit of my studies.

In my original paper (1938), I suggested two ways of calculating the index of intergradation in practice. By one method the following steps are taken. (1) Arrange the data for the two populations compared in a frequency distribution table. (2) From the arranged data in the table construct two overlapping curves representing the two respective populations. (3) Draw a vertical dividing line in the table at a point corresponding to the point of intersection of the two curves, as the heavy vertical line in Tables I and II, the dividing line in our hypothetical example being between the classes 20 and 21. (4) Calculate the percentage of those intergrading specimens that cross the dividing line in each distribution, namely, the specimens to the right of the dividing line in alpha and those to the left of the line in beta, into the total number of specimens in each respective distribution. (5) Add the numbers thus obtained and divide by two. Another way is to tabulate the frequencies in the form of percentage as in Table II, add the smaller of the overlapping frequencies and divide the sum by 2. Applying either method to the assumed data here considered, the index of intergradation shown by the two sets of data is 14 and is of subspecies magnitude, near the lower limit of that magnitude, as previously proposed by me (1938).

The index of divergence may be calculated in two ways complementary to that in which the index of intergradation is calculated, as follows: (1) By reference to Table I, calculate the percentage of the specimens of alpha that are to the left of the dividing line, into the total number of specimens in that sample. Likewise, calculate the percentage of the specimens of beta that are to the right of the dividing line, into the total number of specimens in its sample. Add the two numbers thus obtained and divide by 2. (2) By reference to Table II, add the percentages of the frequencies to the right and to the left of the dividing line, for alpha and beta, respectively, and divide by two. Another way to determine the index of divergence is to first determine the index of intergradation and subtract it from 100. Whichever method is used, the index of divergence of alpha and beta is 86, which is of subspecies magnitude according to the schedule previously proposed by me (1938), near the higher end of that magnitude.

Two Alternative Procedures.—Instead of expressing the index of intergradation as the percentage of the overlapped area into the sum of areas A and B, it may be expressed as the percentage of the overlapped area into one of these areas, namely A, which equals B. In that case the index of intergradation is expressed by the above equation (4), and the step of dividing by 2, as in equation (5), is omitted. The effect of this procedure is to have this index doubled in its numerical value.

Starting with this latter possible index of intergradation, two possible indices of divergence may be proposed.

One method is to propose an index of divergence as represented by the following equation:

Index of divergence = 200—Index of intergradation

$$= 200 - \frac{100\,(a+b)}{A}$$

$$= \frac{100\,[2A-(a+b)]}{A}$$

This possible index of divergence then represents the percentage of the sum of the areas of the two curves diminished by the overlapped area, into one of the curves. The effect of this procedure is to double the numerical value of the index of divergence also, as compared with the procedure originally proposed and discussed above.

The second possible method is to have the index of divergence represented by the following equation:

Index of divergence = 100—Index of intergradation

$$= 100 - \frac{100\,(a+b)}{A}$$

$$= \frac{100\,[A-(a+b)]}{A}$$

This latter possible index of divergence then is represented as the percentage of the area of one of the curves diminished by the overlapped area, or in other words, as the percentage of the area of that part of one curve that is not overlapped, into the area of one curve.

Which one of the above three methods, the one originally employed and the possible two here suggested, is best, is not clear to me at present. Perhaps this could be ascertained by their application to an adequate series of comparable pairs of populations. As these indices merely have a relative, instead of an absolute, value, it is probable that it would not make much difference which one is employed as long as the same one is used consistently throughout

for comparative purposes. The one originally proposed and considered at greater length above seems to be rather more compact and expressive and is perhaps preferable. In reality the three methods are merely modifications of the same basic method. They are all based on a determination, in different ways, of the relation of the overlapped area to the area of one curve or both curves combined.

It is interesting to note that by the use of the original method, the index of intergradation cannot exceed 50, while the index of divergence cannot be less than 50. By the first one of the two alternative methods the index of intergradation runs the gamut of 0 to 100; while the index of divergence cannot be less than 100. By the second of the alternative methods both indices will differ in value from 0 to 100.

Short Cut.—Expressing the frequencies in the form of percentages, as in Table II, is advantageous in that the various numbers concerned in the analysis of Text-fig. 1 may be readily determined directly from the table. Thus, the value of a equals the sum of the values in alpha that are located to the right of the dividing line which in the hypothetical example equals 9. The value of b equals the sum of the values in beta to the left of the dividing line, namely, $12.7 + 5.4 = 18.1$. The numerical value of the part of the curve that is not overlapped equals $100-(a + b) = 100-(9 + 18.1) = 72.9$.

Acknowledgment.—I wish to express my grateful appreciation to Arthur Schach and to Ralph P. Silliman who read successive trial versions of the manuscript and made constructive suggestions for its clarification.

Literature Cited

Ginsburg, Isaac

1937. The species and its subdivisions. Copeia, 1937 (3): 184-188.

1938. Arithmetical definition of the species, subspecies and race concept, with a proposal for a modified nomenclature. Zoologica, 23 (3): 253-286.

1939. The measure of population divergence and multiplicity of characters. Jour. Washington Acad. Sci., 29 (8): 317-330.

1940. Divergence and probability in taxonomy. Zoologica, 25 (1): 15-31.

1944. A description of a new gobiid fish from Venezuela, with notes on the genus Garmannia. Jour. Washington Acad. Sci., 34 (11): 375-380.

1950. Review of the western Atlantic Triglidae (Fishes). Texas Jour. Sci., 2 (4): 489-527.

1951. Western Atlantic tonguefishes with descriptions of six new species. Zoologica, 36 (3): 185-201.

1952. Eight new fishes from the Gulf coast of the United States, with two new genera and notes on geographic distribution. Jour. Washington Acad. Sci., 42 (3): 84-101.

1953. Western Atlantic scorpionfishes. Smithsonian Misc. Coll., 121 (3): 1-103.

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Contents

5

Biometry of Seven Natural Populations of the Platyfish, *Xiphophorus maculatus*, from Central America

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(Text-figures 1-15)

THE Central American platyfish, *Xiphophorus maculatus* (Guenther), a small viviparous cyprinodont, is found in each of the seven major Atlantic coastal river systems from southern Mexico to British Honduras. The northernmost limit of its range is the Río Jamapa near the city of Veracruz, and its southernmost habitat is in the Belize River.

The seven populations of the platyfish, a freshwater species, are geographically isolated. Their migration along the Atlantic coast is not now possible, for the species does not tolerate sea water. Owing to the relative smallness of the species—specimens rarely reach two inches under natural conditions—there is considerable difficulty in obtaining accurate measurements for the purpose of comparing one group with another. Nevertheless, the present paper is concerned with an attempt to use some of the standard methods in distinguishing the members of seven geographically isolated river populations on the basis of three body measurements and proportions: length and depth of body and length of caudal peduncle. Other measurements of the body proper were made but found not to be useful. In a later study, the analysis of the data on the frequencies of number of dorsal fin rays in similar platyfish populations will be presented.

It has been found by the authors, Gordon (1947) and Gordon & Gordon (1950, 1954), that the seven populations of the highly polymorphic platyfish are distinguishable on the basis of the gene frequencies for twelve heritable melanic patterns. These twelve black markings are produced by two genetically different kinds of melanophores, one small, the other large. Seven black patterns, on and near the tail, produced by micromelanophores, are controlled genetically by a series of seven dominant autosomal multiple alleles. Five additional spotted patterns on the body produced by macromelanophores are controlled by a series of five dominant sex-linked multiple alleles. In each series, the unmarked platyfish is the universal recessive. On the basis of the frequencies of the twelve dominant patterns and their universal recessive, the authors (1954) worked out a "taxonomic key" to identify each of the river races, providing at least 200 platyfish in a population are available.

Other genetical analyses of the Central American platyfish and related species and their hybrids by Gordon (1948, 1950, 1951a, b) have revealed that the macromelanophore genes are potentially injurious. For example, in fish with certain genetic recombinations, the large black pigment cells grow atypically. This leads to the development of a pathological state of melanosis or of melanoma. Since about 20% of all feral platyfish have at least one large black pigment cell pattern, it was thought desirable to determine if there exist any correlations between the various macromelanophore patterns and size and body proportions.

Material and Methods

The northernmost population of the platyfish, *Xiphophorus maculatus*, was taken near the mouth of the Río Jamapa, in the central part of the state of Veracruz, just south of the city of Veracruz (see Text-fig. 1). To the south, another population was found in the Río Papaloapan, about 100 miles inland along the border of the state of Oaxaca. The third population was taken from various localities in the Río Coatzacoalcos

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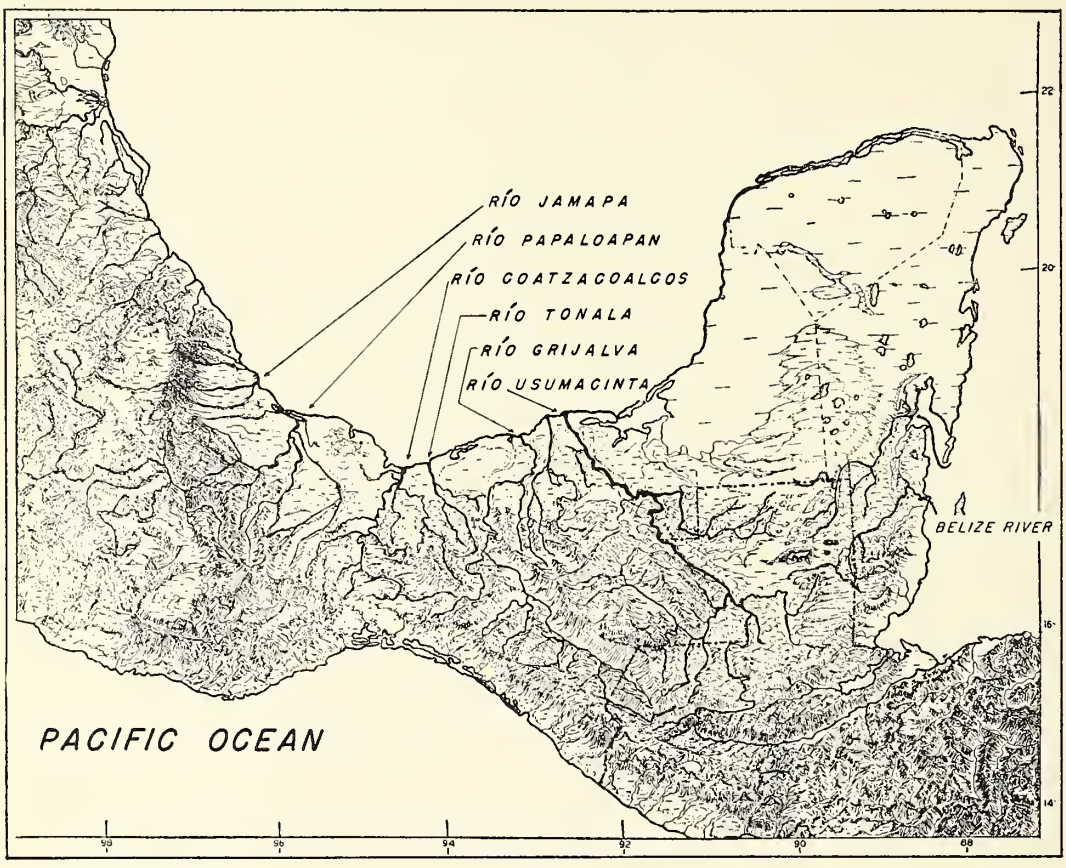


Text-fig. 1. Part of Mexico, Guatemala and British Honduras showing the seven river systems in which *Xiphophorus maculatus* were taken. The platyfish found in the Río Jamapa were taken within 10 miles of the mouth. The platyfish from the Río Grijalva were found within 20 miles of the mouth. The localities at which platyfish were taken in the other five river systems are shown in subsequent figures. The arrow above the Belize River indicates the location of two creeks of independent drainage from which platyfish were taken; the locations of these and other stations in British Honduras are shown in Text-fig. 5. This map is modified from that of Hoy, 1943.

in southern Veracruz. A fourth group was located in a small stream, Arroyo de la Venta, that entered the Río Tonalá near its mouth at the border of the states of Veracruz and Tabasco. Another large population was collected in the lowland area of the Río Grijalva near the capital of Tabasco, Villa Hermosa. The sixth major platyfish group was taken from the Lake Petén area and other parts of the upper Río Usumacinta in Guatemala. Small numbers of the species were found in the region of the mouth of the Río Hondo, the river that separates Mexico from British Honduras, but a large representative collection (the seventh) was taken from a tributary near the mouth of the Belize River just north of Belize.

The platyfish taken in the field were preserved in 10% formalin. In the laboratory they were washed in water and transferred to and stored in 70% alcohol. The specimens showed little or no shrinkage and their melanic patterns remained intact. Although nearly 9,000 platyfishes were collected, only 2,993 were measured for this analysis, of which 2,517 were males and 476 were females. The collections utilized are listed in Table 1 and their localities are shown in Text-figs. 1, 2, 3, 4 and 5. A summary of some genetic differences between the seven platyfish populations is presented diagrammatically in Text-fig. 6.

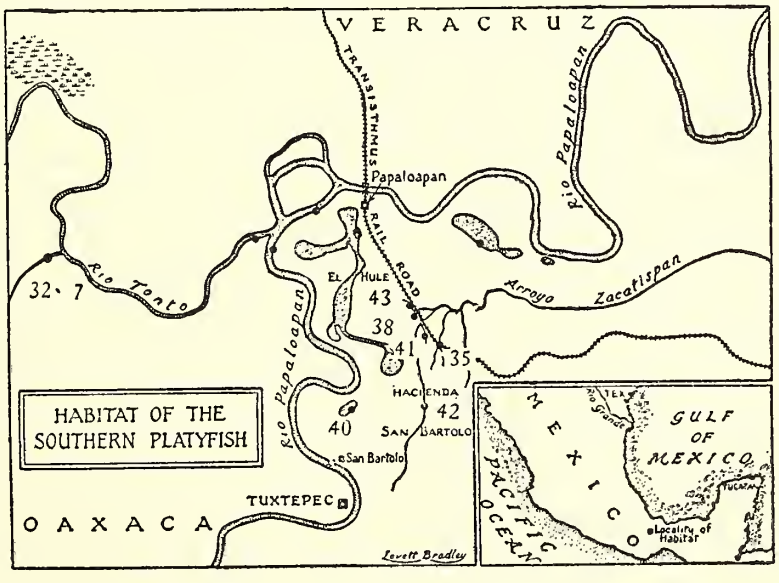
Three measurements were made, using dividers and a millimeter scale, and were recorded in millimeters: (1) *Standard Length,* the distance from the tip of the snout to the end of the vertebral column; (2) *Depth,* the distance from the base of the anterior margin of the dorsal fin to the midpoint between the origins of the pelvic fins; and (3) *Length of Caudal Peduncle,* the

TABLE 1. COLLECTIONS OF *Xiphophorus maculatus* UTILIZED IN THIS STUDY

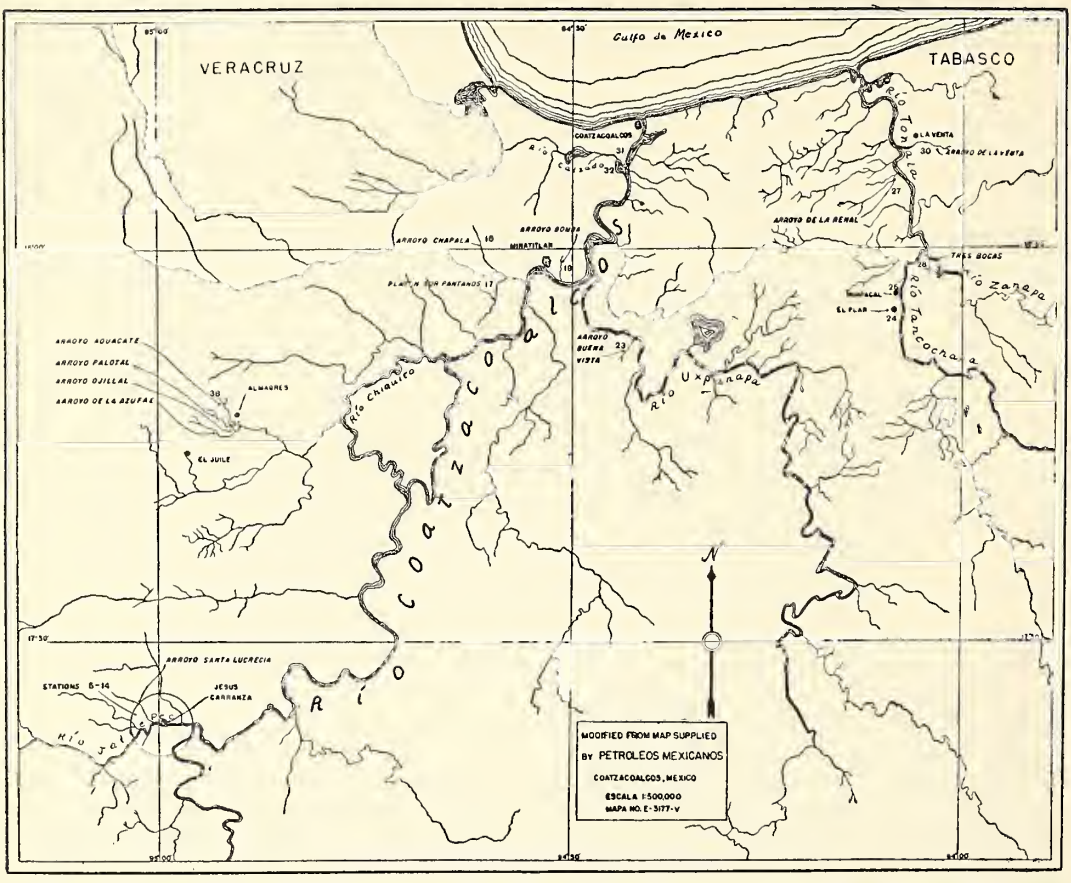
River	Collectors	Year	Field Numbers	Number of Males Measured	Depository	Location Shown in Text-fig.
Jamapa	Myron Gordon, Evelyn Gordon, James W. Atz	1939	45a, b	346	UMMZ[1]	1
Papaloapan	Same	1939	43a, b, c, d 38a, b, c	1331	UMMZ	2
Coatzacoalcos	Myron Gordon, James W. Atz, F. G. Wood, Jr.	1948	GAW 35, 17, 23, 32, 31	485	NYZS[2]	3
Tonalá	Same	1948	GAW 24, 25, 27, 28, 29, 30	81	NYZS	3
Grijalva	Myron Gordon, Jesus Garcia	1952	GG 12	50	NYZS	1
Usumacinta	Carl L. Hubbs, Henry van der Schalie, Josslyn Van Tyne	1935	M35-8 to M35-135	176	UMMZ	4
Belize	Myron Gordon, Gerald Fairweather	1949	GF 5	48	NYZS	5
			Total	2517		

[1] UMMZ–University of Michigan, Museum of Zoology.
[2] NYZS–New York Zoological Society.

distance from the base of the anterior margin of the anal fin to the end of the vertebral column (Text-fig. 7). In platyfish from the Río Tonalá samples, the caudal peduncle was measured from the midpoint between the origins of the pelvic fins to the end of the vertebral column instead of in the above manner. In order to compare these populations with the others properly, the measurements had to be made to correspond. A sliding scale was therefore constructed, giving the difference in the two measurements of caudal peduncle length as a function of the length of the fish. Since the correlation between standard length and the difference between the two measurements is large ($z = .96 \pm .12$), the results obtained are only slightly inaccurate. The orig-



TEXT-FIG. 2. The collection stations in the vicinity of Papaloapan, Oaxaca, at which *Xiphophorus maculatus* were taken. The numbers on the map are the original numbers of the 1939 collection except that 32-7 indicates the station at which a collection was made in 1932. Platyfish were taken at each station except station 42.



TEXT-FIG. 3. Map of the Río Coatzacoalcos and Río Tonalá systems showing the stations at which *Xiphophorus maculatus* were taken. The numbers on the map are the original numbers of the 1948 collection with the prefix GAW omitted. Platyfish were not taken at some of the stations shown.

inal measurements are used in comparison within the Río Coatzacoalcos system, but the corrected measurements are used in the comparisons between river populations.

Two form indices were calculated from these data: the *depth index*, equal to the standard length divided by the depth, and the *length of caudal peduncle index*, equal to the standard length divided by the length of the caudal peduncle.

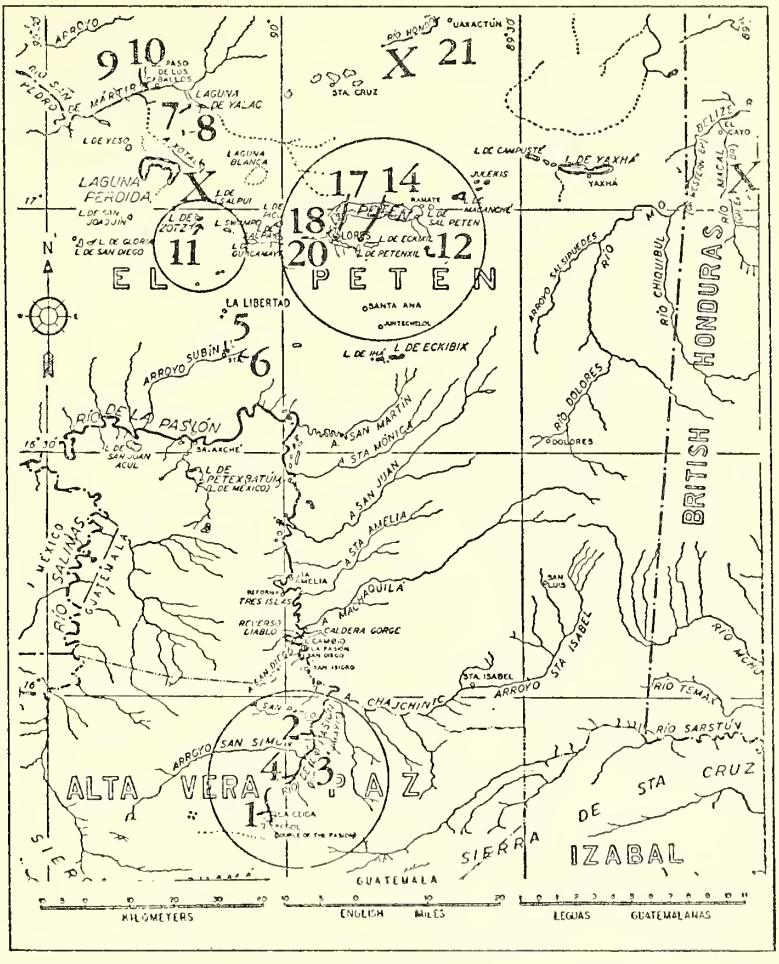
Means, standard deviations and standard errors of means were calculated for these data. A modified formula of Simpson & Roe (1939) for the standard error of the difference between two means was used in the calculation of the critical ratio for determination of the significance of this difference:

$$\frac{d}{\sigma_d} = \frac{M_1 - M_2}{\sqrt{\frac{\sigma_1^2}{N_2} + \frac{\sigma_2^2}{N_1}}}$$

The criterion of significance was taken as $d/\sigma_d = 2$. It should be noted that use of the above formula determines whether the observed difference in means is consistent with the hypothesis that the samples came from populations with the same mean and variance.

As an aid to visualizing the result of these comparisons, the data were graphed by a modification of the method described by Dice & Leraas (1936). In these graphs, the long vertical line represents the range of observations; the center line of the rectangle represents the arithmetic mean, and the upper and lower limits of the rectangle represent the mean plus and minus twice its standard error. A significant difference should be indicated by non-overlapping of rectangles; this is not always the case, however, because of varying sample sizes, and hence no precise conclusions can be based on the graphs. Sample sizes, given in the tables, have sometimes been omitted from the graphs for the sake of clarity.

An attempt was made to adapt the method



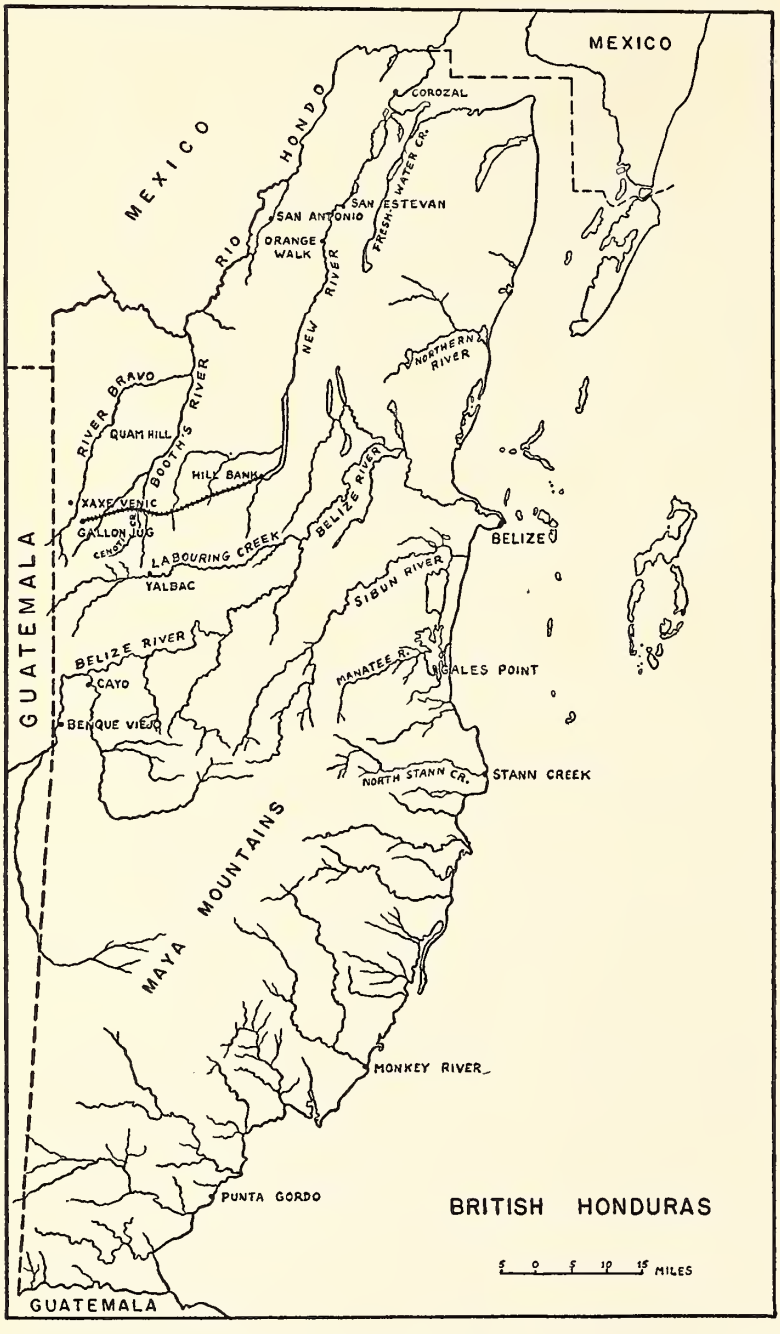
TEXT-FIG. 4. The collection stations in the Guatemala portion of the Río Usumacinta system at which *Xiphophorus maculatus* were taken. The numbers on the map indicate stations where platyfish were taken. The Xs indicate stations where *Xiphophorus helleri* were taken. This map is modified from that prepared by Hubbs & van der Schalie, 1937. (Reprinted from Gordon, 1947).

described by Parr (1949) for use in comparing the various populations, by taking advantage of the correlation between standard length and depth index (see below). Because of the difficulty in evaluating the significance of the differences between regression coefficients, and because the results seemed more contradictory and less accurate than those derived by the earlier procedure, the method was not used.

The question of whether there is any sexual dimorphism in the proportions of the body, aside from being of interest in itself, is important in determining whether data on both sexes may be combined in making interpopulation comparisons.

It was found impractical to dissect or section the many specimens to determine their sex. It was decided therefore, that any fish, whether completely mature or not, which showed a conspicuous andromorphic modification of the anal fin was to be classified as a male. The remaining fish were classified as female or immature, according to size and female-like body contours. On the basis of some preliminary tests, it was found that for comparative purposes a measurement of an adequate number of males was superior to a measurement of females and immature fish. As a consequence, comparisons between local populations within the Río Papaloapan, Río Coatzacoalcos and Río Usumacinta systems, as well as comparisons between each of the seven river populations taken as wholes, were made only between males. In the Río Grijalva and Belize River collections, only about 50 males were measured. The other five populations had already been analyzed, and the results indicated that no advantage, in interpopulation comparisons, would be gained by measuring more than fifty specimens from any one population.

To determine the relationship of macromelanophore pattern to size and body proportions, the members of the Río Jamapa population were classified on the basis of appropriate criteria, Tables 2, 3. Although platyfish have five macromelanophore patterns, only three of them are found in the Río Jamapa population



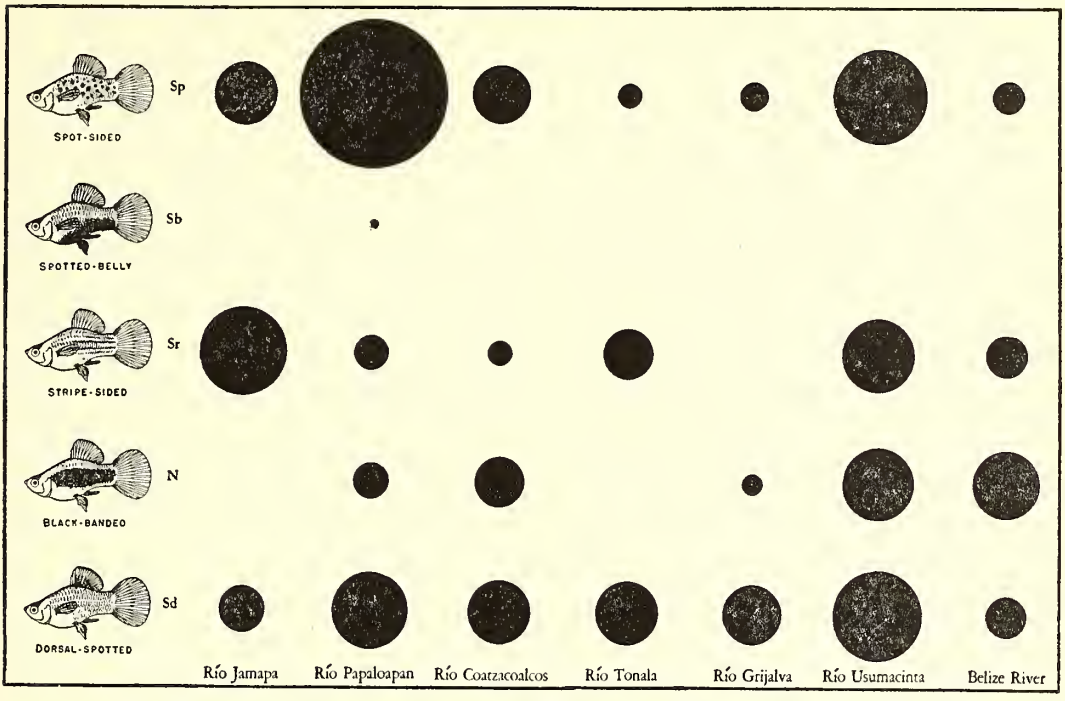
TEXT-FIG. 5. Map of British Honduras. The representative collection of *Xiphophorus maculatus* was taken just north of the city of Belize. Smaller collections were taken just north of Corozal, at San Antonio, at Hill Bank and at San Estevan.

(Text-fig. 6). These three are *Spot-sided, Sp; Spotted-dorsal, Sd;* and *Stripe-sided, Sr.* The absence of a pattern is designated by the plus sign, "+", the universal recessive. On the basis of the results obtained from the analysis of the Río Jamapa platyfish, it was decided that it was not advantageous to carry out this type of analysis for the platyfish of the remaining six populations.

ANALYSIS OF DATA

Sexual Dimorphism

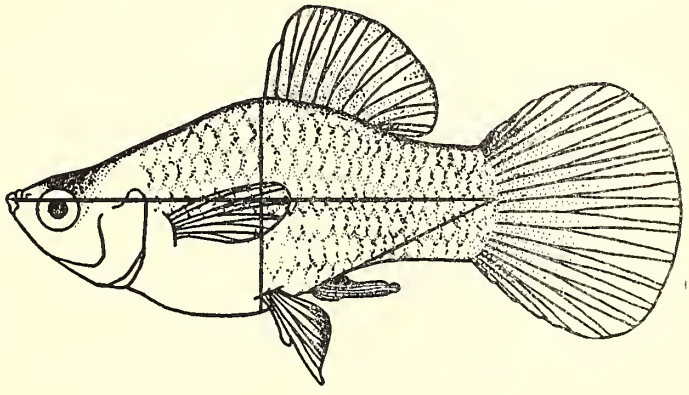
The mean standard length, depth index and length of caudal peduncle index, together with the standard errors of these means and the corresponding standard deviations, for the Río Jamapa males, are given in Table 2, and for the Río Jamapa females in Table 3. The ratio of the difference in mean standard length between



TEXT-FIG. 6. The macromelanophore patterns and their relative gene frequencies in seven river populations of *Xiphophorus maculatus*. The area of the circle for each population and pattern is proportional to the gene frequency in that population of the allele producing that pattern. For convenience in printing, the circle for *Sb* is drawn about four times its proper area. Missing circles indicate zero gene frequencies. Each of the seven river populations has a distinct set of macromelanophore gene frequencies. This graph is taken from Gordon & Gordon (1954).

males and females to its standard error is 0.8. The corresponding ratio for the depth index is 1.0, and that for the length of caudal peduncle index is 15.0. The length of caudal peduncle index is thus significantly greater in females than in males.

To eliminate the possible inclusion of immature specimens in comparing males with females, 50 females and 36 males, selected for their large size, were compared. Table 4 and Text-fig. 8 present the data obtained on these fish. No significant difference in length or depth index was found. As before, the females have a significantly larger length of caudal peduncle index. Since no significant difference was found in standard length, this result indicates a probable difference in the absolute length of the caudal peduncle. To test this hypothesis, the mean length of caudal peduncle for males was compared with that for females. The caudal



TEXT-FIG. 7. The measurements which were made on specimens of *Xiphophorus maculatus*. The horizontal line indicates the *standard length*. The vertical line indicates the *depth*. The diagonal line indicates the *length of caudal peduncle*. This figure represents a male.

peduncle was found to be significantly longer, 1.1 ± .2 mm, in males than in females. An explanation of this difference, on the basis of differential growth in the sexes, will be suggested in the discussion.

Comparison of Fish with Different Macromelanophore Patterns

The results of comparing the measurements of fish having various macromelanophore patterns are presented in Table 2 for males and Table 3 for females, Text-figs. 9, 10 and 11. Significant differences were found (1) between the values for all males and those that are "+" (recessive) with respect to standard length; (2) between all males and *Sr* males with respect to depth indcx; and (3) between "+" males and *Sr* males with respect to standard length and depth index. No significant differences were found between any other groups, either males or females.

Comparison of Fish Collected at the Same Place on Different Days of the Same Week

The mean values of the standard length, depth index and length of caudal peduncle index for males obtained in four collections, '39-43a, b, c and d, made on March 4, 6, 7, 10, 1939, from the same pool, are presented in Table 5 and Text-fig. 12. The males collected on March 4 are significantly longer than those in each of the other three days' collections. The fish collected on March 6 are longer than those collected on March 10. The individuals in the collection of March 10 have a significantly greater depth index than those in the other collections; those in the collection of March 7 have a greater depth index than those in the collection of March 4. The fish collected March 10 have a lower length of caudal peduncle index than those in the other collections. No other significant differences were noted.

Table 2. Analysis of Differences between Males of Differing Phenotypes in the Río Jamapa Population of *Xiphophorus maculatus*

Pattern	N		M	σ_M	σ	$d/\sigma_d(\Sigma)$	$d/\sigma_d(+)$
+	292	S.L.	23.8	.218	3.723	5.4	
		S.L./D.	2.36	.008	.137	.9	
		S.L./L.C.P.	2.10	.006	.102	1.2	
Sr	35	S.L.	25.6	.412	2.439	.5	2.8
		S.L./D.	2.25	.018	.107	4.3	4.6
		S.L./L.C.P.	2.12	.012	.071	.5	1.1
Sd	9	S.L.	24.8	.576	1.728	.5	.8
		S.L./D.	2.27	.028	.084	1.8	2.0
		S.L./L.C.P.	2.11	.026	.077	.0	.3
Sp	10	S.L.	25.8	.967	3.058	.5	1.7
		S.L./D.	2.40	.035	.111	1.1	1.0
		S.L./L.C.P.	2.15	.021	.066	1.4	1.5
All Males	346	S.L.	25.3	.177	3.287		5.4
		S.L./D.	2.35	.007	.130		.9
		S.L./L.C.P.	2.11	.006	.111		1.2

Explanation of symbols used above:
N = Number of specimens.
M = Mean.
σ = Standard deviation.
σ_M = Standard error of mean.
S.L. = Standard Length.
S.L./D. = Standard Length divided by Depth.
S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.
d/σ_d (Σ) = The ratio of the difference between the mean for the pattern and the mean for all males to this difference's standard error.
d/σ_d (+) = The corresponding ratio for + males instead of all males.

Comparison of Local Populations

A comparison between collections made at two nearby points in the Río Papaloapan is presented in Table 6. No significant differences were found.

Data for five collections from the Río Coatzacoalcos are given in Table 7 and Text-fig. 13. With regard to standard length, the only outstanding collection is GAW 32, which was significantly larger than the other populations collected. GAW 31, collected in the same general locality, has the smallest mean length and is significantly different from three of the other four collections examined. Most of the populations examined are significantly different from each other in the depth index. The only significant differences in the length of caudal peduncle index are between populations GAW 17, GAW 23 and population GAW 35.

The Río Tonalá sample was too small to permit comparisons within this river system.

Gordon (1947) found that the three populations in the Río Usumacinta system were genetically distinct with respect to the frequencies of melanophore pattern genes. Since the samples were too small to permit comparisons between individual stations, the samples were combined according to these groups and then compared. The data for the Río Usumacinta system are given in Table 8 and Text-fig. 14. In the comparisons of standard length, Group A (Laguna de Zotz) was significantly different from both Group B (Río de la Pasíon) and Group C (Laguna de Petenxil-Petén). Groups B and C were not significantly different. All three groups are significantly different in the depth index. Group A is significantly different in the length of the caudal peduncle index from Group B, but not from C. Group B is significantly different from C with respect to this index.

Comparison of the Seven River Systems

It will be found that the number of specimens from each river system shown in Table 9 differs from the total of the numbers for the local populations in that system. This is because samples too small to be used for local comparisons are included here. The comparative values are shown in Table 9 and Text-fig. 15.

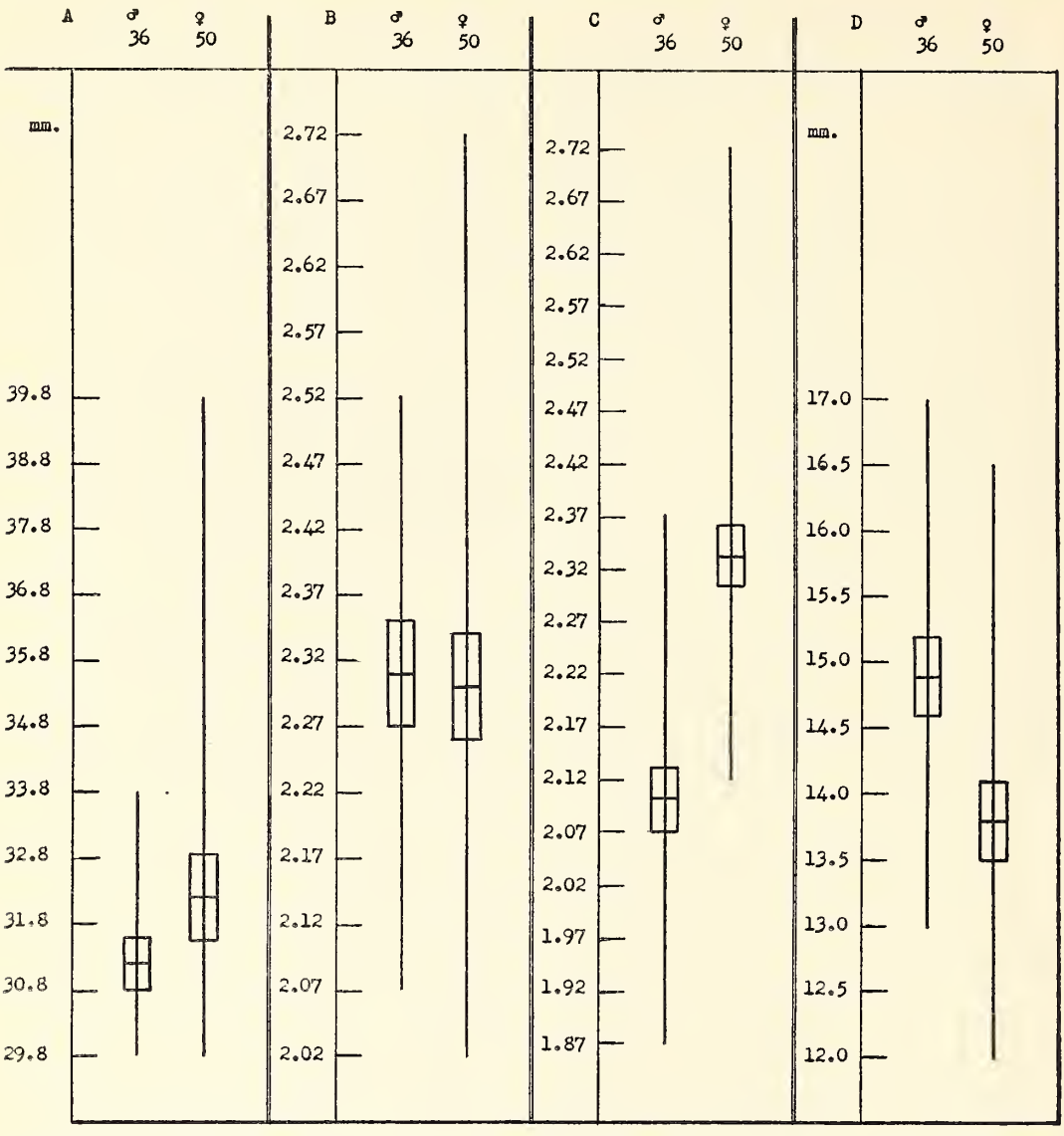
1. With respect to standard length, the following observations may be made. The Río Jamapa specimens are significantly longer than all others. The Río Tonalá collection is not significantly different from either that of the Río Coatzacoalcos or that of the Río Usumacinta, its mean lying midway between their significantly differing means. The Río Grijalva specimens are not different from those of the

Table 3. Analysis of Differences between Females of Differing Phenotypes in the Río Jamapa Population of *Xiphophorus maculatus*

Pattern	N		M	σ_M	σ	$d/\sigma_d(\Sigma)$	$d/\sigma_d(+)$
+	435	S.L.	25.4	.179	3.723	.4	
		S.L./D.	2.32	.007	.146	.2	
		S.L./L.C.P.	2.23	.006	.125	.0	
Sr	35	S.L.	25.6	.489	2.893	.2	.3
		S.L./D.	2.34	.020	.118	.0	.8
		S.L./L.C.P.	2.24	.017	.100	.5	.5
Sd	1	S.L.	26.0			.1*	.1*
		S.L./D.	2.60			1.7*	1.9*
		S.L./L.C.P.	2.26			.3*	.2*
Sp	5	S.L.	27.6	1.431	3.205	1.2	1.3
		S.L./D.	2.37	.020	.045	.4	.8
		S.L./L.C.P.	2.21	.057	.128	.4	.3
All Females	476	S.L.	25.5	.169	3.686		.4
		S.L./D.	2.34	.007	.153		.2
		S.L./L.C.P.	2.23	.005	.109		.0

The symbols used above are explained in Table 2, except that d/σ_d (Σ) in this table involves all females instead of all males.

* Only one *Sd* female was collected. Accordingly, the numbers given are ratios of the deviation of this female from the mean for the group with which it is being compared to the standard deviation for that group.



TEXT-FIG. 8. Sexual dimorphism in the largest specimens of *Xiphophorus maculatus* in the Río Jamapa collection. The sections of the graph headed A, B, C and D refer to the standard length, depth index, length of caudal peduncle index and length of caudal peduncle, respectively. The graph is based on data on 36 males and 50 females; these data are given in Table 4. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean.

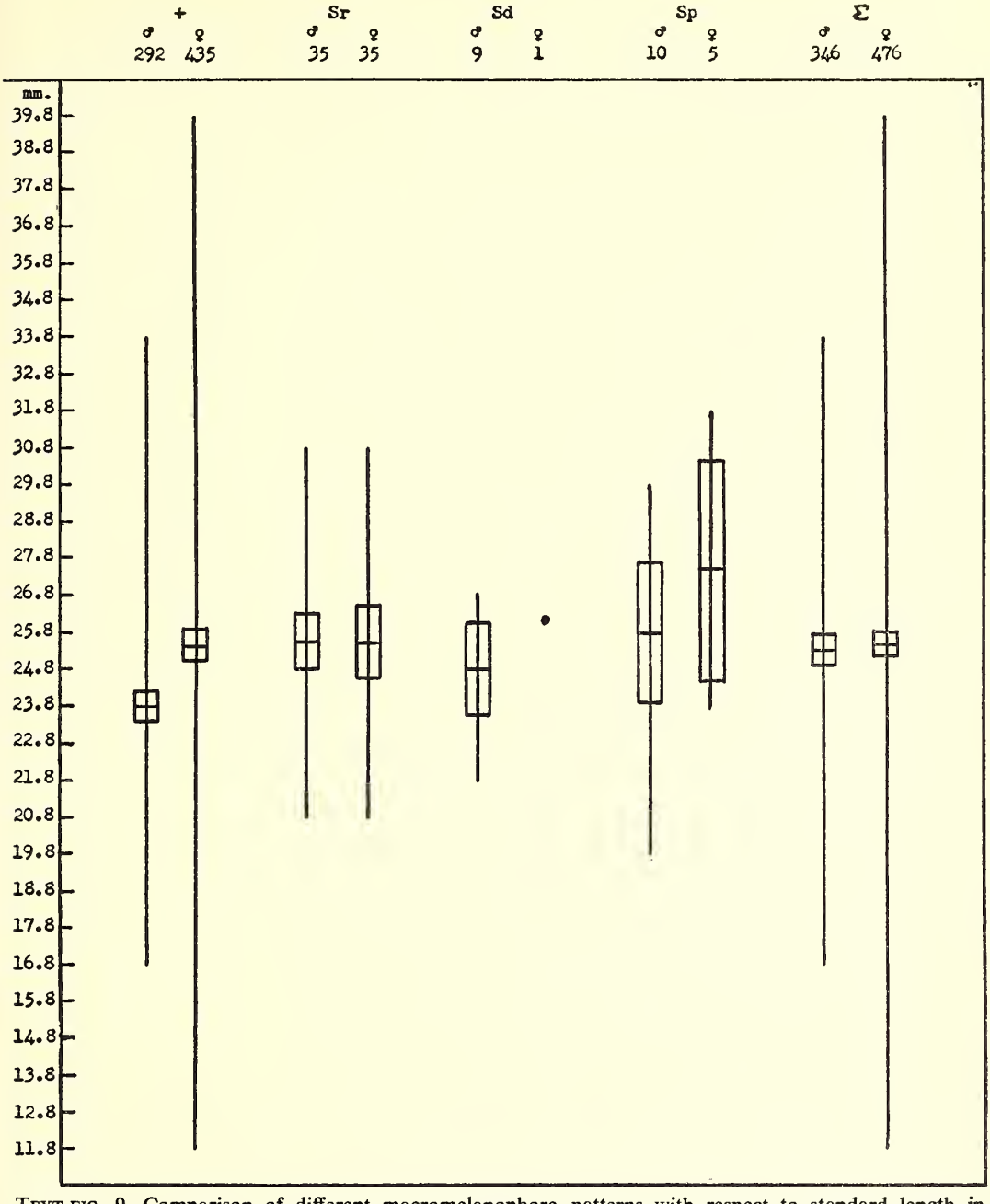
Río Coatzacoalcos and the Río Tonalá. The Belize River specimens are not significantly different from those of the Río Coatzacoalcos, Río Tonalá and Río Usumacinta. All other means showed significant differences.

2. In comparisons involving the depth index, the Río Tonalá population is found to be not significantly different from that of the Río Papaloapan or the Río Grijalva population from the Río Jamapa one. All other differences are significant.

3. The Río Grijalva specimens are not significantly different in the length of caudal peduncle index from the Belize River fish. All other differences are significant.

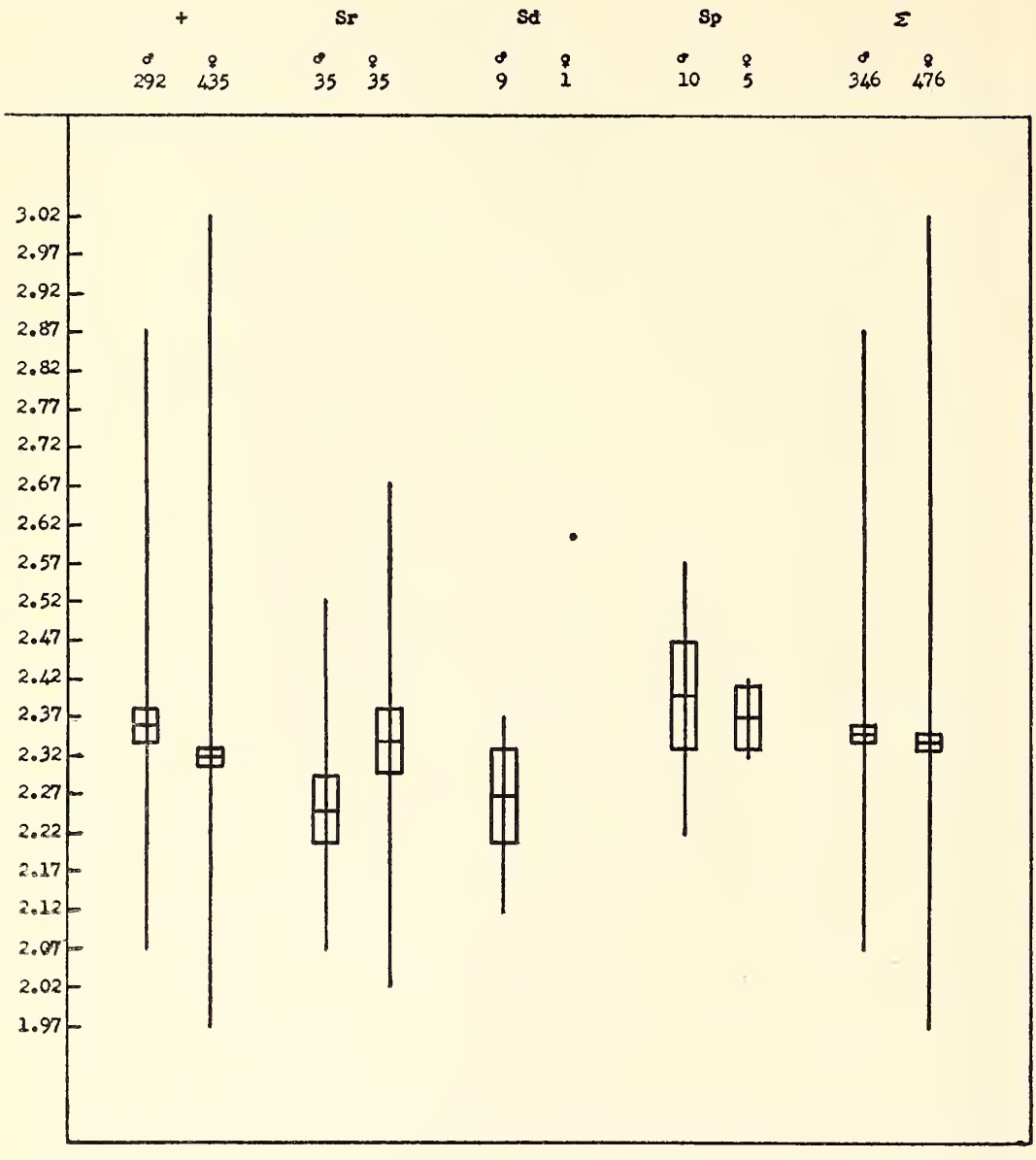
Correlation Between Standard Length and Length of Caudal Peduncle Index

Table 10 gives the correlation between standard length and the length of caudal peduncle index for each of the seven river populations. Only the Río Grijalva population shows a sig-



TEXT-FIG. 9. Comparison of different macromelanophore patterns with respect to standard length in *Xiphophorus maculatus*. The data are for the Río Jamapa sample and are given in Tables 2 and 3. The symbol Σ designates the sum of all phenotypic groups. The numbers near the top are the sample sizes. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean.

nificant correlation; $z = -.35 \pm .15$. Of the seven correlation coefficients, five are negative. The mean value of the seven coefficients is $-.086 \pm .049$. The value of χ^2 for determining the possibility of all seven correlations arising simultaneously by chance is 11.40 (with seven degrees of freedom); the probability of a worse fit is about 0.12. The correlation coefficient for



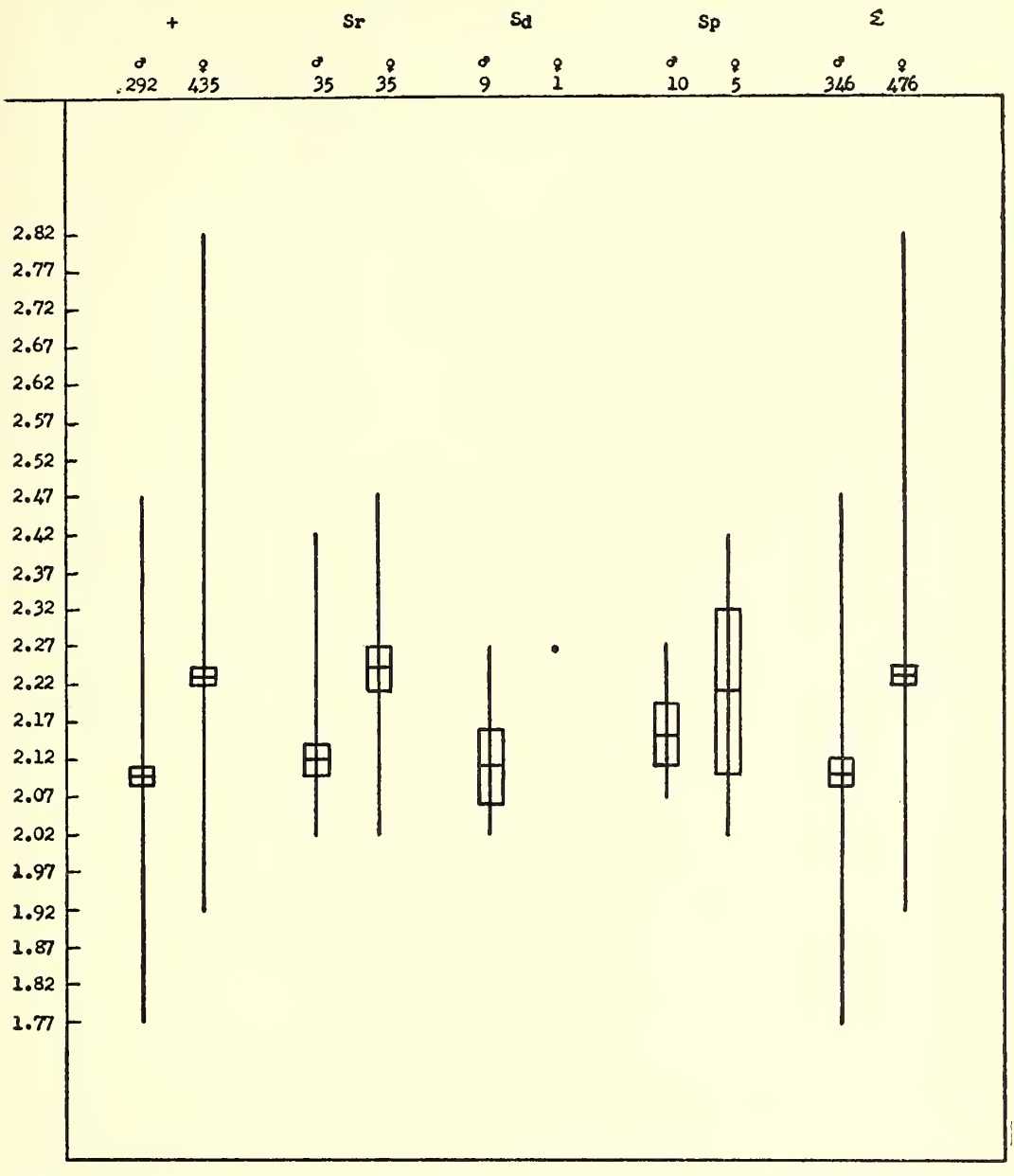
TEXT-FIG. 10. Comparison of different macromelanophore patterns with respect to depth index in *Xiphophorus maculatus*. The data are for the Río Jamapa sample and are given in Tables 2 and 3. The symbol Σ designates the sum of all phenotypic groups. The numbers near the top are the same sizes. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean.

the Río Grijalva population differs significantly from those for the Río Tonalá and Río Usumacinta ones. No other significant differences are found among the correlation coefficients.

DISCUSSION

Sexual Dimorphism

Cohen (1946) found the mean length of *Xiphophorus maculatus* females significantly larger than that of males. This difference between his results and those obtained here may be due to the small number of specimens he used (seven of each sex) or to the fact that he used domesticated stock, raised under aquarium conditions. The difference in length accounts for the fact that he gives significantly different values of the depth index for males and females (3.61 and 2.82, respectively; see below).

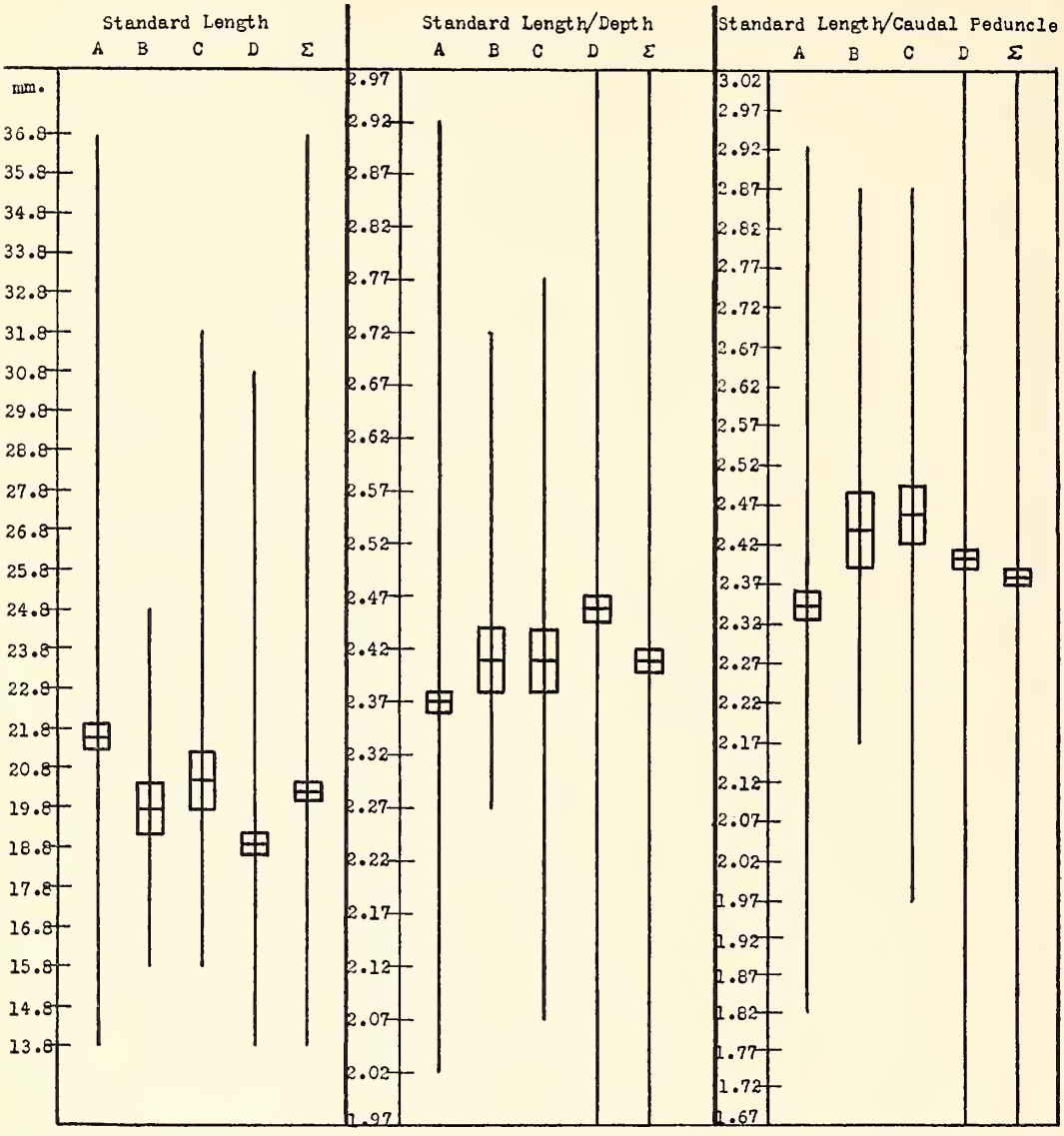


TEXT-FIG. 11. Comparison of different macromelanophore patterns with respect to length of caudal peduncle index in *Xiphophorus maculatus*. The data are for the Río Jamapa sample and are given in Tables 2 and 3. The symbol Σ designates the sum of all phenotypic groups. The numbers near the top are the sample sizes. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean.

Bellamy (1922), on the other hand, obtained the same depth index for both males and females (2.7).

Our data indicate that males and females from the Río Jamapa do not differ in mean standard length nor in mean depth index. The caudal peduncle, however, is 1.1 mm (average) longer in males than in females. This is based upon fish that are 32 mm (average) in length.

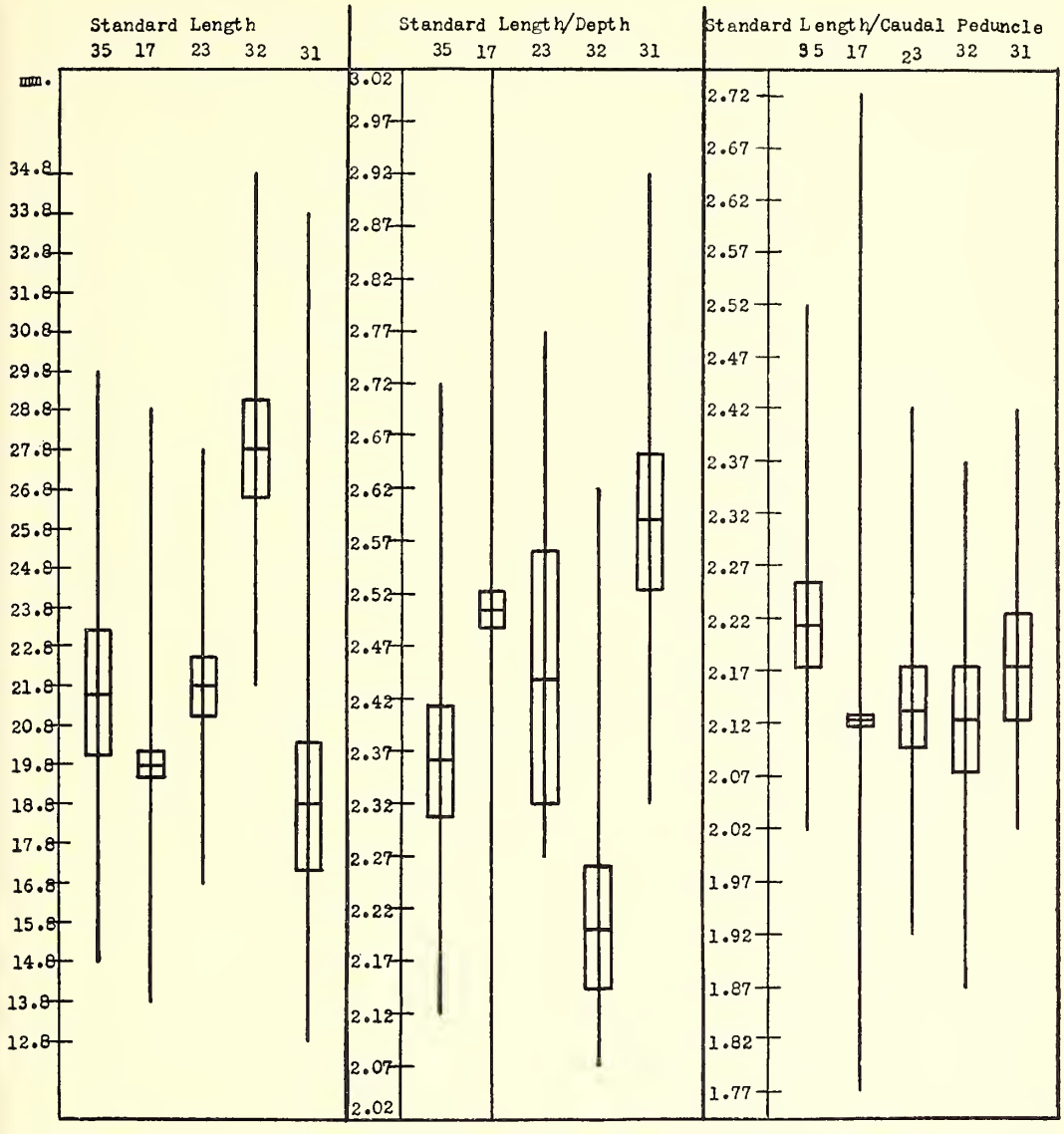
The difference in length of the caudal peduncle between males and females may be attributed to fact that during morphogenesis of the male gonopodium, the anal fin moves to a more forward position (Gordon & Benzer,



TEXT-FIG. 12. Comparison of *Xiphophorus maculatus* taken from the same pool on different days of the same week. A, B, C and D indicate the collections made at station '39-43 on March 4, 6, 7 and 10, 1939, respectively. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean. The data on which this graph is based are given in Table 5.

1946). This realignment produces a relatively longer caudal peduncle in the male and a corresponding dissimilarity in the indices.

Since males differ from females with respect to the length of the caudal peduncle, it is probably better to use members of one sex in making comparisons between populations. In deciding which sex to use, it should be noted that males may be distinguished from females and immature fish by the andromorphic modifications of their anal fin. Females can, as a practical matter, be distinguished from immature fish only approximately on the basis of their size and their body contours. For this reason it is advantageous to use males in interpopulation comparisons. The use of males also eliminates the possibility of errors arising from the inclusion of temporarily deep-bodied gravid females. Accordingly, all comparisons between populations in the present analysis are based on males alone.

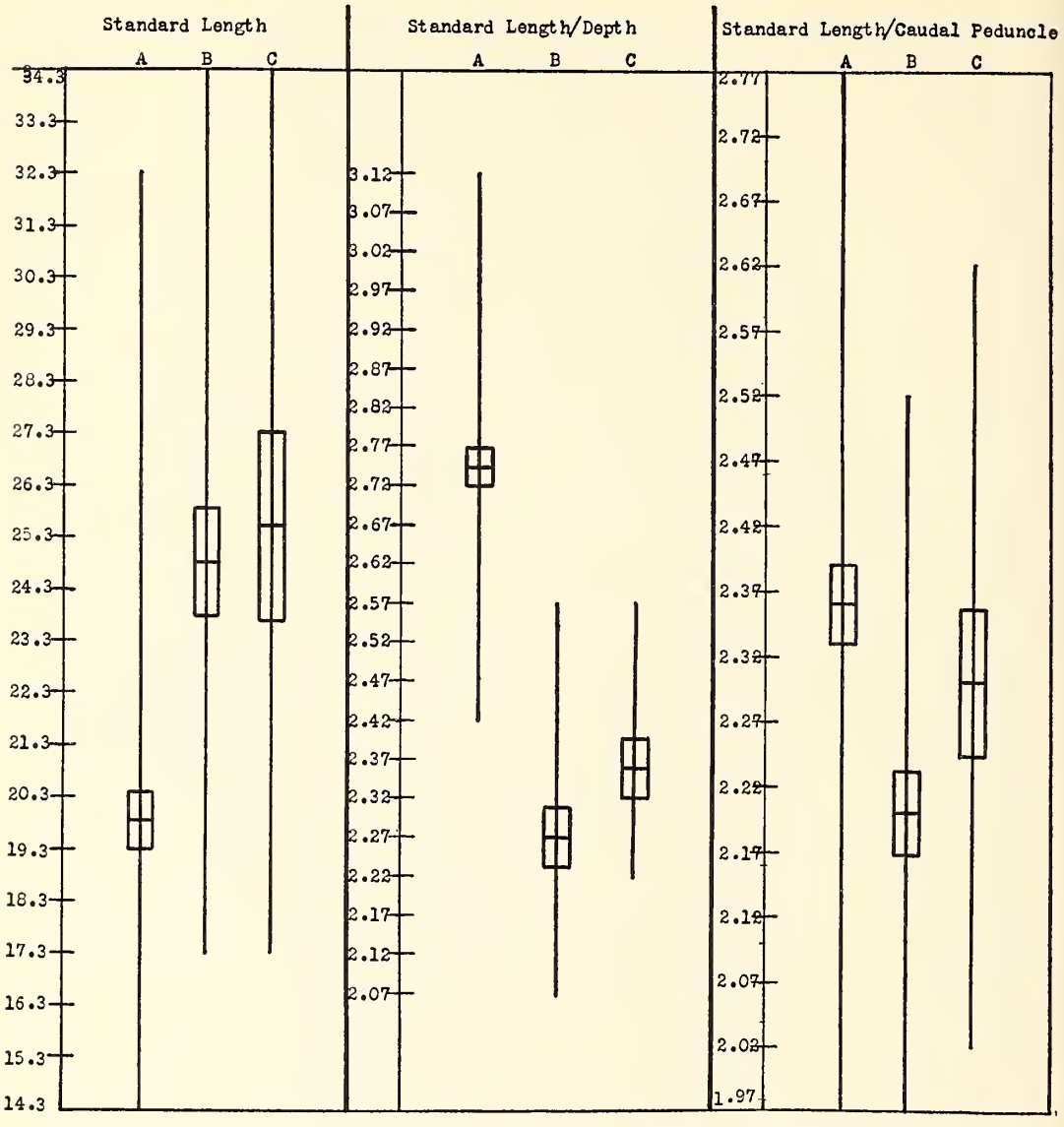


TEXT-FIG. 13. Comparison of five local populations of *Xiphophorus maculatus* in the Río Coatzacoalcos. The numbers near the top are station numbers. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean. The data on which this graph is based are given in Table 7.

Comparison of Fish of Different Macromelanophore Patterns

In comparing individuals of different phenotypes, males and females were considered separately. Thus the difficulties of considering both sexes at once are eliminated. The possibility that size differences between fish of different phenotypes may exist in one sex only is also not overlooked.

Males without a macromelanophore pattern (+) appear to be shorter than males marked with a macromelanophore pattern. This difference, however, probably results from the inclusion of immature fish. The macromelanophore patterns develop in fish only as these fish reach a size comparable to that at which they mature. Accordingly, a macromelanophore pattern may not appear in a fish until after the modification of its anal fin has become sufficient for the fish to be classified as a male. Certain of the fish classified as "+" males would, therefore, have been expected to develop macromelanophore



TEXT-FIG. 14. Comparison of three population groups of *Xiphophorus maculatus* in the Río Usumacinta. A, B and C refer to the Laguna de Zotz, Río de la Pasión and Laguna de Petenxil-Petén, respectively. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean. The data on which this graph is based are given in Table 8.

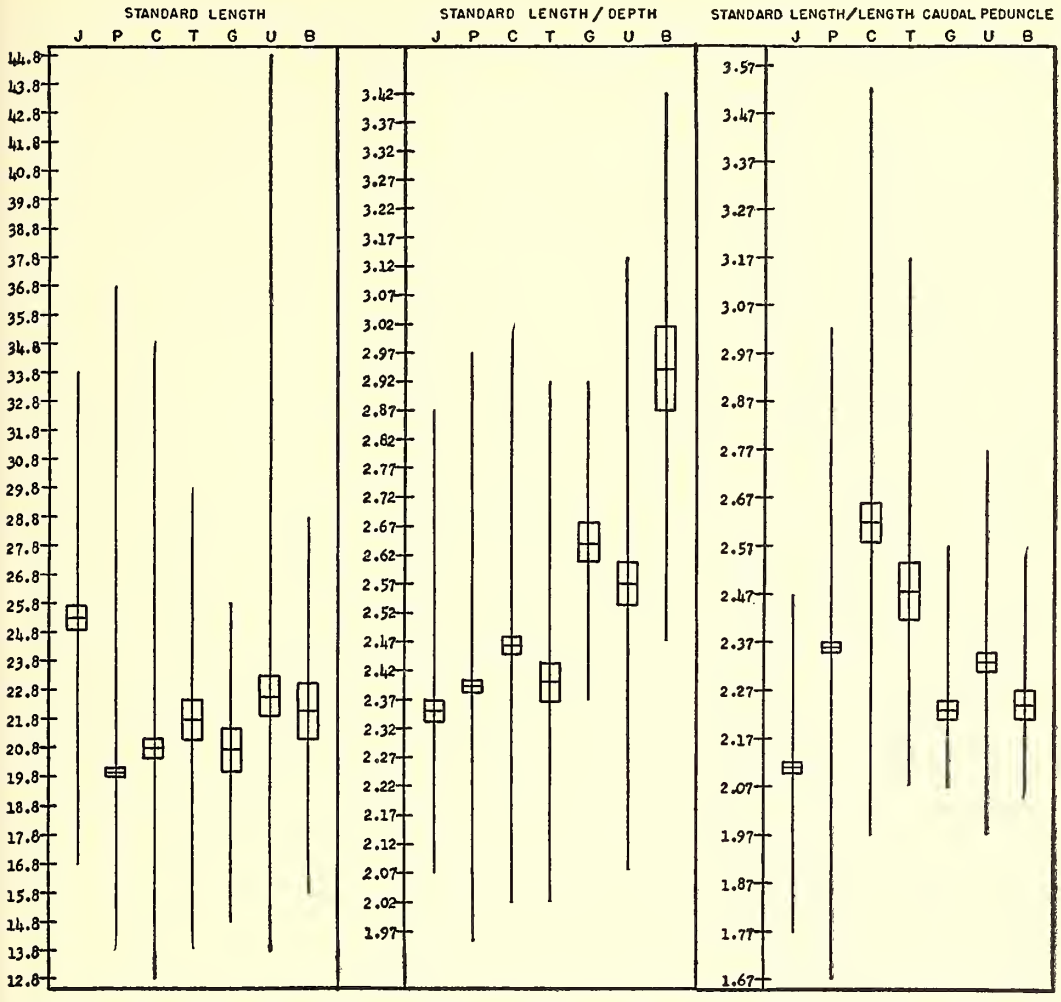
patterns if they had been permitted to live. For this reason the mean standard length of "+" males might be expected to be less than that of males with other phenotypes.

The separation of females from immature fish is based primarily on size. Fish large enough to be called females have, in general, developed their macromelanophore patterns. For this reason any difference in length between "+" females and females with macromelanophore patterns is minimized.

The difference in depth index between *Sr* males and "+" males is probably a consequence of the fact that the *Sr* males are longer in mean than the "+" males, together with the tendency for longer fish to be relatively more deep (see below).

The absence of differences in size between the fish of different phenotypes permits one to combine all phenotypes in comparing populations.

One method by which the polymorphism, and



TEXT-FIG. 15. Comparison of seven river populations of *Xiphophorus maculatus*. J, P, C, T, G, U and B refer to the Río Jamapa, Río Papaloapan, Río Coatzacoalcos, Río Tonala, Río Grijalva, Río Usumacinta and Belize River, respectively. The long vertical lines indicate observed ranges; the rectangles indicate twice the standard error above and below the mean. The data on which this graph is based are given in Table 9.

in particular the diversity of macromelanophore pattern, of the platyfish might be maintained is through selection. The data indicate that any selection which may be present operates in a manner which neither causes, nor is caused by, substantial size differences. Since only a weak selection pressure is sufficient to maintain polymorphism, differences in size too small to be detected by the present analysis may occur and yet have significance with regard to selection. On the other hand, since selection is not necessarily associated with size differences, the data here considered can at best have merely negative significance with regard to determining whether selection is present.

Direct Comparison of Natural Populations with Respect to Size and Shape

The difficulty in comparing natural populations of platyfish by means of measurements is not one of finding characteristics which differ from population to population. The question is rather how to find dimensions and indices that reflect permanent characteristics of the populations.

The '39-43 collection is valuable for determining the validity of using any particular dimensions and indices as means of distinguishing populations. Statistically significant differences are found between the fish taken on different days with respect to both the depth index and the

length of caudal peduncle index, as well as with respect to length. Since all fish taken at station '39-43 represent the same population, it is clear that differences found in either of these indices between two samples do not reflect genetic differences within the populations from which the samples were drawn. The indices for a population apparently may change appreciably within a week. Accordingly, if the indices can be used at all to distinguish populations, they must be used with caution.

TABLE 4. ANALYSIS OF DIFFERENCES BETWEEN THE LARGEST MALES AND FEMALES OF A COLLECTION OF *Xiphophorus maculatus* TAKEN IN THE RÍO JAMAPA

Sex	N		M	σ_M	σ	d/σ_d
♂	36	S.L.	31.2	.195	1.170	.2
		S.L./D.	2.31	.019	.114	.3
		S.L./L.C.P.	2.10	.015	.090	9.6
		L.C.P.	14.9	.164	.984	6.4
♀	50	S.L.	32.2	.325	2.298	
		S.L./D.	2.30	.020	.141	
		S.L./L.C.P.	2.33	.017	.120	
		L.C.P.	13.8	.142	1.004	

Explanation of symbols used above:
N = Number of specimens.
M = Mean.
σ = Standard deviation.
σ_M = Standard error of mean.
S.L. = Standard Length.
S.L./D. = Standard Length divided by Depth.
S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.
The column headed d/σ_d gives the ratio of the difference between the mean for females and that for males to its standard error.

The variations in length and in the indices from day to day in the population at station '39-43, whatever their causes, must be considered in comparing populations. Nevertheless, an attempt was made to explain the variation; a knowledge of the causes of the differences might make it possible to devise effective methods of comparing populations. The mean standard length of the fish collected decreased from day to day. Gordon (1947) said that predatory birds and fishes eliminated relatively more of the larger platyfish between the four collections. The pool that held the platyfish was shallow and the water was rapidly evaporating. On the last collection from the pool all fish species were represented mainly by their young.

The differences found among the various days' collections would be expected if there were negative correlation between the depth index and the standard length, and between the length of caudal peduncle index and the standard length. The appropriate correlation coefficients were therefore examined. In the case of the depth index vs. standard length correlation, the 75 males from the Río Usumacinta give a value of the correlation coefficient $z = -.51 \pm .12$. Seventy-five males from the Río Tonalá also

TABLE 5. BIOMETRIC COMPARISON[1] OF FOUR COLLECTIONS OF *Xiphophorus maculatus* MADE FROM THE SAME POOL[2] IN THE RÍO PAPALOAPAN WITHIN A WEEK

Day of Collection	Number of Specimens		Mean	Standard Deviation	Ratio of Difference in Mean to Its Standard Error as Compared with March 6	March 7	March 10
March 4, 1939	473	S.L.	21.3±1.75	3.82	3.4	2.5	12.1
		S.L./D.	2.35±.006	.136	2.0	2.5	10.0
		S.L./L.C.P.	2.32±.008	.163	4.1	6.2	5.5
March 6, 1939	48	S.L.	19.4±.293	2.08		1.5	2.1
		S.L./D.	2.39±.017	.117		.0	3.2
		S.L./L.C.P.	2.42±.024	.166		.7	1.9
March 7, 1939	85	S.L.	20.2±.361	3.34			.5
		S.L./D.	2.39±.014	.129			2.6
		S.L./L.C.P.	2.44±.018	.167			1.7
March 10, 1939	404	S.L.	18.6±.122	2.53			
		S.L./D.	2.44±.007	.130			
		S.L./L.C.P.	2.38±.005	.156			

[1] The following are used in the comparison:
S.L. = Standard Length.
S.L./D. = Standard Length divided by Depth.
S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.
[2] Station number '39-43.

TABLE 6. BIOMETRIC COMPARISON[1] OF TWO LOCAL POPULATIONS OF *Xiphophorus maculatus* FROM THE RÍO PAPALOAPAN

Collection Number	Number of Specimens		Mean	Standard Deviation	Ratio of Difference in Mean to Its Standard Error as Compared with '39-43
'39-38	321	S.L.	20.1±.254	3.51	.5
		S.L./D.	2.39±.008	.149	0.0
		S.L./L.C.P.	2.34±.009	.153	1.4
'39-43	1010	S.L.	20.0±.110	3.51	
		S.L./D.	2.39±.005	.174	
		S.L./L.C.P.	2.36±.005	.166	

[1] The following are used in the comparison:
S.L. = Standard Length.
S.L./D. = Standard Length divided by Depth.
S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.

give $z = -.51 \pm .12$. Scatter diagrams indicated that similar values would be obtained for the other populations. In view of the good correlation in the Río Usumacinta and Río Tonalá collections and the scatter diagrams, it seemed unnecessary to make any further calculations. It may be concluded that as a fish grows larger, it grows relatively deeper.

Whether there is any correlation between the length of caudal peduncle and standard length is not clear. Since no definite results with preliminary methods were obtained, correlation coefficients were calculated for all seven river populations (Table 10). The coefficients obtained differ significantly among themselves. It is possible that the correlation coefficient obtained from any given sample depends on the mean length and range in length of the fish in the sample. Thus the differences in correlation coefficients among the samples may

TABLE 7. BIOMETRIC COMPARISON[1] OF FIVE LOCAL POPULATIONS OF *Xiphophorus maculatus* FROM THE RÍO COATZACOALCOS

Collection Number	Number of Specimens		Mean	Standard Deviation	Ratio of Difference in Mean to Its Standard Error as Compared with GAW 17	GAW 23	GAW 32	GAW 31
GAW 35	31	S.L.	21.6±.799	4.45	3.2	.2	6.1	2.5
		S.L./D.	2.36±.026	.174	5.0	1.1	4.0	5.9
		S.L./L.C.P.	2.21±.018	.103	3.6	2.7	3.0	1.7
GAW 17	321	S.L.	19.8±.152	2.72		4.4	15.8	1.8
		S.L./D.	2.50±.008	.321		1.9	10.0	3.1
		S.L./L.C.P.	2.12±.002	.135		.4	0.0	1.9
GAW 23	40	S.L.	21.8±.373	2.36			9.0	3.9
		S.L./D.	2.44±.059	.373			3.1	2.0
		S.L./L.C.P.	2.13±.022	.137			.3	1.2
GAW 32	26	S.L.	27.8±.586	2.99				9.4
		S.L./D.	2.20±.029	.150				9.7
		S.L./L.C.P.	2.12±.024	.121				1.5
GAW 31	29	S.L.	18.8±.734	3.95				
		S.L./D.	2.59±.028	.153				
		S.L./L.C.P.	2.17±.023	.125				

[1] The following are used in the comparison:
S.L. = Standard Length.
S.L./D. = Standard Length divided by Depth.
S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.

not reflect any essential differences among the populations. Combining all the data either by using χ^2 or by taking a mean value gives a probability of about 0.1 of obtaining as much or more correlation by chance. In view of this low, although not significantly low, probability and the significant correlation obtained from the Río Grijalva sample, it may well be that, at least in some populations, there is negative correlation between the standard length and the length of the caudal peduncle index.

The local populations within each river system and the seven river systems may be compared by the means of the indices of the length of caudal peduncle. As shown above, the correlation between it and the standard length, if there is any at all, is small. It may also be noted that collections GAW 31 and GAW 32, which were made at nearby points in the Río Coatzacoalcos, do not differ significantly for this index, although they show a great difference (9 mm) in standard length. Again, in the Río Usumacinta region, the group collections from the Laguna de Zotz and those from the Laguna de Petenxil-Petén, which would be expected to resemble each other to some degree because of their geographical proximity, show a non-significant difference only for this index.

On the other hand, the March 4, 1939, collection from the Río Papaloapan differs significantly with respect to this index from the other three collections made within a week from the same population. It may be noted that Gordon & Benzer (1945) found that the differences which exist in vertebral counts between the seven species of xiphophorin fishes occur in the region of the caudal peduncle. The length of caudal peduncle index is probably better than the standard length or depth index for comparing populations. Its validity for this purpose is nevertheless doubtful; if it can be used at all, it must be used with considerable caution.

With these various limitations in mind, the following comparisons of local groups may be presented.

1. There are no significant differences between two representative Río Papaloapan populations.

2. In the Río Coatzacoalcos populations, considering only the length of caudal peduncle index, the only significant differences occur between collection GAW 35 and collections GAW 17, GAW 23 and GAW 32. These differences may reflect geographical factors, such as distance or altitude. GAW 35 was taken at a place somewhat removed from those where the other three collections were made (Text-fig. 4). It should be noted that none of the differences is very great, however.

3. In the populations from the upper Río Usumacinta region, the Río de la Pasión group is significantly different from the Laguna de Zotz and Laguna de Petenxil-Petén groups with respect to the length of caudal peduncle index, but the latter two groups do not differ significantly from each other. This may be accounted for by the fact that the Laguna de Zotz and the Laguna de Petenxil-Petén are located more closely to each other.

With similar limitations upon significance of the differences found, the following comparisons between the seven river populations taken as

Table 8. Biometric Comparison[1] of Three Local Populations of *Xiphophorus maculatus* from the Río Usumacinta System

Location	Number of Specimens		Mean	Standard Deviation	Ratio of Difference in Mean to Its Standard Error as Compared with Río de la Pasión	Ratio of Difference in Mean to Its Standard Error as Compared with Laguna de Petenxil-Petén
Laguna de Zotz	94	S.L.	19.9±.305	2.96	8.7	7.3
		S.L./D.	2.74±.013	.130	12.6	7.4
		S.L./L.C.P.	2.36±.016	.152	6.5	1.7
Río de la Pasión	51	S.L.	24.8±.507	3.62		.7
		S.L./D.	2.27±.021	.148		2.7
		S.L./L.C.P.	2.20±.017	.120		3.2
Laguna de Petenxil-Petén	25	S.L.	25.5±.931	4.65		
		S.L./D.	2.36±.021	.106		
		S.L./L.C.P.	2.30±.028	.139		

[1] The following are used in the comparison:
S.L. = Standard Length.
S.L./D. = Standard Length divided by Depth.
S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.

TABLE 9. BIOMETRIC COMPARISON[1] OF SEVEN RIVER POPULATIONS OF *Xiphophorus maculatus*

River	Number of Specimens		Mean	Standard Deviation	Ratio of Difference in Mean to Its Standard Error as Compared with Río Papaloapan	Río Coatzacoalcos	Río Tonalá	Río Grijalva	Río Usumacinta	Belize River
		S.L.	25.3±.177	3.29	65.5	18.2	9.9	9.4	7.5	6.4
Jamapa	346	S.L./D.	2.35±.007	.130	4.4	10.3	2.9	1.4	13.7	25.6
		S.L./L.C.P.	2.11±.006	.111	28.0	39.6	21.7	7.4	18.9	7.5
		S.L.	20.0±.030	1.11		7.1	10.9	4.0	16.7	10.7
Papaloapan	1331	S.L./D.	2.39±.004	.155		8.3	.5	11.3	13.4	23.5
		S.L./L.C.P.	2.36±.004	.156		27.8	6.2	5.8	2.4	5.2
		S.L.	20.8±.167	3.68			2.0	.2	5.1	2.1
Coatzacoalcos	485	S.L./D.	2.46±.008	.165			3.0	3.3	6.6	18.2
		S.L./L.C.P.	2.62±.010	.222			5.2	12.3	16.0	11.7
		S.L.	21.7±.389	3.50				1.7	1.5	.4
Tonalá	81	S.L./D.	2.40±.018	.162				17.8	5.8	14.9
		S.L./L.C.P.	2.48±.032	.292				5.9	5.4	5.4
		S.L.	20.7±.400	2.80					2.6	2.0
Grijalva	50	S.L./D.	2.64±.017	.116					8.9	7.7
		S.L./L.C.P.	2.23±.012	.082					4.5	.4
		S.L.	22.6±.362	4.81						.8
Usumacinta	176	S.L./D.	2.57±.018	.238						3.3
		S.L./L.C.P.	2.33±.011	.151						3.8
		S.L.	22.0±.507	3.52						
Belize	48	S.L./D.	2.94±.036	.249						
		S.L./L.C.P.	2.24±.017	.120						

[1] The following are used in the comparison:
S.L. = Standard Length.
S.L./D. = Standard Length divided by Depth.
S.L./L.C.P. = Standard Length divided by Length of Caudal Peduncle.

wholes may be considered. The differences in standard length between the river populations show no clear linear geographical trend. The depth index increases from north to south in an orderly manner; the trend is perfect except for two cases in which neighboring populations are reversed. Thus, platyfish become relatively deeper from south to north. The length of caudal peduncle index seems to increase southward from the Río Jamapa to the Río Coatzacoalcos and then to decrease from the Río Coatzacoalcos to the Belize River.

Other Possible Methods of Comparing Populations

Various procedures have been considered in comparing the populations to avoid the difficulties and uncertainties mentioned above. For example, consideration was given to the possibility that only fish of a certain length, i.e., range in length, be used in comparing populations. All specimens not of the length chosen would be ignored. One of the disadvantages of this method is the difficulty of obtaining a sufficient number of specimens of the same length. Even if enough specimens of one size are obtained, there would be an objection to the validity of the procedure. The selection of certain specimens from each population might result in specimens which were among the largest in their population being compared with others which were among the smallest in theirs. This procedure thus might produce differences in the indices that do not reflect the real differences in the populations.

Another procedure for comparing populations that was considered is to examine the rectilinear regression coefficients. If the relation between a given dimension and the fish's length is linear, the regression coefficients would be unaffected by the lengths of the specimens in a sample being studied. In that case, a comparison of regression coefficients is a valid method of comparing populations. There is, however, no reason to suppose that the requisite linearity exists. If a dimension is not linearly related to length, the regression of that dimension on length will be affected by the lengths of the specimens in the sample, i.e., two samples of different mean length from the same population may have different regression coefficients. Accordingly, the regression coefficients are not necessarily more reliable than dimensions and indices in comparing populations. In this connection, Schaefer (1952) used the study of regression lines to compare yellowfin tunas, *Neothunnus macropterus*, from Hawaiian waters with those from the American West Coast. He noted, however, that the regression analysis was "beset with difficulties" and found that only because the magnitude of the differences between populations was sufficient, was he able to obtain results.

TABLE 10. CORRELATION BETWEEN STANDARD LENGTH AND STANDARD LENGTH DIVIDED BY LENGTH OF CAUDAL PEDUNCLE IN *Xiphophorus maculatus*

	r	z	σ_z	z/σ_z
Río Jamapa	—.141	—.14	.12	1.2
Río Papaloapan	—.159	—.16	.12	1.3
Río Coatzacoalcos	—.066	—.07	.12	.6
Río Tonalá	+.144	+.15	.12	1.0
Río Grijalva	—.335	—.35	.15	2.3
Río Usumacinta	+.044	+.04	.12	.4
Belize River	—.090	—.09	.15	.6

General Uniformity of the Male Platyfish in Each of the Seven River Systems

The male platyfish in each of the seven river populations do not differ in mean standard length from 22.7 mm by more than 12%; in mean depth index from 2.65 mm by more than 11%; or in mean length of caudal peduncle index from 2.37 mm by more than 11%. These percentages are equivalent to about 2 mm in length and about 1 mm in depth or length of caudal peduncle. It may be noted that the platyfish is also uniform in size with respect to comparisons between fish of different sex and between those of different macromelanophore pattern. The decrease in relative depth from north to south very likely reflects a real difference between the populations. Aside from this trend, the data indicate that it is the uniformity, rather than the diversity between isolated populations that is significant.

Although platyfish are uniform as to size and shape, the seven river populations can all be recognized as distinct on the basis of the frequencies of their melanic patterns (Gordon & Gordon, 1954). Thus it may be said that the meristic criteria used in this study for distinguishing populations of platyfish are not as precise as those based upon their inherited color patterns.

SUMMARY

The platyfish, *Xiphophorus maculatus*, has been collected from seven major rivers which empty into the Atlantic Ocean from Mexico to British Honduras. Measurements were made on a total of 2,993 adult platyfish from these seven river systems with reference to their standard length and two body indices, namely, the standard length divided by the depth of body and the standard length divided by the length of caudal

peduncle. Males and females were compared separately, since it was found that the length of caudal peduncle was slightly longer in males than in females.

No significant differences were found in the body measurements and proportions between fish with various inherited macromelanophore patterns, or fish without these patterns. Since polymorphism in platyfish is probably maintained by a weak selection pressure, differences in size too small to be detected by the present analysis may occur and yet have significance with regard to selection. On the other hand, since selection is not necessarily associated with size differences, the data here considered can at best have only negative significance with regard to determining whether selection is present.

Statistically significant differences were found in standard length and in the body indices among four collections of adult male platyfish taken from the same pool on four different days within one week. In view of this great variability, the measurements employed seem inadequate for the purpose of differentiating the seven populations of platyfish. However, the mean relative depth of the platyfish appears to decrease progressively from north to south. The other measurements do not follow this trend.

The seven isolated populations of platyfish are essentially similar with regard to their size and body proportions. Because of this, the meristic criteria used in this study in an attempt to distinguish the seven populations are not as precise as those based upon the frequencies of their inherited color patterns.

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References

Bellamy, A. W.

1922. Breeding experiments with the viviparous teleosts, *Xiphophorus helleri* and *Platypoecilus maculatus* (Günth.). Anat. Rec., 23: 98-99. (Abstract).

Cohen, Herman

1946. Effects of sex hormones on the development of the platyfish, *Platypoecilus maculatus*. Zoologica, 31: 121-128.

Co., Inc., New York, 414 pp.

Dice, L. R. & H. J. Leraas

1936. A graphic method for comparing several sets of measurements. Cont. Lab. Vert. Genetics, Univ. Michigan, No. 3: 1-3.

Gordon, Hugh & Myron Gordon

1950. Colour patterns and gene frequencies in natural populations of a platyfish. Heredity, 4: 61-73.

1954. Maintenance of polymorphism by potentially injurious genes in seven natural populations of the platyfish, *Xiphophorus maculatus*. Jour. Genetics (in press).

Gordon, Myron

1947. Speciation in fishes. Distribution in time and space of seven dominant multiple alleles in *Platypoecilus maculatus*. Advances in Genetics, 1: 95-132.

1948. Effects of five primary genes on the site of melanomas in fishes and the influence of two color genes on their pigmentation. Biol. of Melanomas, Spec. Publ., N. Y. Acad. Sci., 4: 216-268.

1950. The origin of modifying genes that influence the normal and atypical growth of pigment cells in fishes. Zoologica, 35: 19-20. (Abstract).

1951a. The variable expressivity of a pigment cell gene from zero effect to melanotic tumor induction. Cancer Research, 11: 676-686.

1951b. Genetics and correlated studies of normal and atypical pigment cell growth. Growth, Symposium, 10: 153-219.

Gordon, Myron & Paul Benzer

1945. Sexual dimorphism in the skeletal elements of the gonopodial suspensoria in xiphophorin fishes. Zoologica, 30: 57-72.

Hoy, H. E.

1943. Physiographic diagram of Mexico. Papers, Michigan Acad. Sci., 28: 441-443.

Hubbs, C. L. & Henry van der Schalie

1937. Map of El Petén, Guatemala, and bounding regions of British Honduras and Mexico (Chiapas, Tabasco, and Campeche). Univ. Michigan, Ann Arbor.

Parr, A. E.

1949. An approximate formula for stating taxonomically significant proportions of fishes with reference to growth changes. Copeia, 1949: 47-55.

Schaefer, M. B.

1952. Comparison of yellowfin tuna of Hawaiian waters and of the American west coast. U. S. Fish and Wildlife Service, Fish. Bull. No. 72, 52: 353-373.

Simpson, George G. & Anne Roe

1939. Quantitative Zoology. McGraw-Hill Book

6

A Survey of the Poisonous Fishes of Johnston Island[1]

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(Text-figure 1)

Introduction

THIS paper is the second of a series of epidemiological reports concerning the poisonous fishes of various island areas in the tropical Pacific. The first report by the present authors (1953) dealt with the poisonous fishes of the Phoenix Islands. For a general résumé of the over-all problem of poisonous fishes and fish poisoning the reader is referred to two earlier papers (Halstead, 1951, 1953).

The problem of poisonous fishes represents one of the neglected fields of medical and ichthyological research. The existing confusion and lack of precise data regarding the identity, geographical distribution and biology of toxic fishes and the nature of ichthyosarcotoxins can only hamper the economic development of the shore fisheries of the tropical Pacific. The fact that a species of fish may be commercially valuable in one locality and violently toxic in another can be a major factor in outlawing otherwise valuable fishing grounds. Future world demands for protein food sources will necessitate a more rigid control and efficient utilization of the vast food reserves of the ocean, and consequently the problem of poisonous marine organisms will become of increasing importance. One of the primary objectives of the present series of investigations is to provide accurate epidemiological data on toxic fishes.

During February, 1951, Dr. Vernon E. Brock and his associates of the Division of Fish and Game for the Territory of Hawaii made a fisheries survey of Johnston Island. The study revealed that poisonous fishes were a local public health menace. A small collection of reef fishes was captured, shipped to Loma Linda for analysis and subsequently found to be toxic. Plans for a more comprehensive study were formulated at the suggestion of Colonel Robert T. Cronau, USAF, who at that time was the Base Commander for Johnston Island. During May, 1951, the senior author made a preliminary survey in order to determine the collecting conditions, availability of small boat transportation and the probable incidence of ichthyosarcotoxism. In October and November, 1951, the junior author made a more complete field study and collected most of the specimens upon which the present report is based.

Incidence and Clinical Characteristics of Fish Poisoning at Johnston

Johnston Island was uninhabited until 1909, when it was leased from the Territory of Hawaii by a private guano company (Bryan, 1942). The project did not succeed and the operation was soon closed. In 1926 the island was made a bird reservation. In 1934 it was placed under the jurisdiction of the United States Navy, but military personnel did not inhabit the island until about the latter part of 1939. Prior to 1939 there were no "permanent human residents," consequently there is only meager information regarding the edibility of the fish fauna before the arrival of the military. Mr. Edwin H. Bryan, Jr., of the Bernice P. Bishop Museum in Honolulu, who has maintained voluminous files on the Pacific islands for many years, believes that fish poisoning at Johnston Island is of recent origin. Mr. Bryan was a member of the scientific staff

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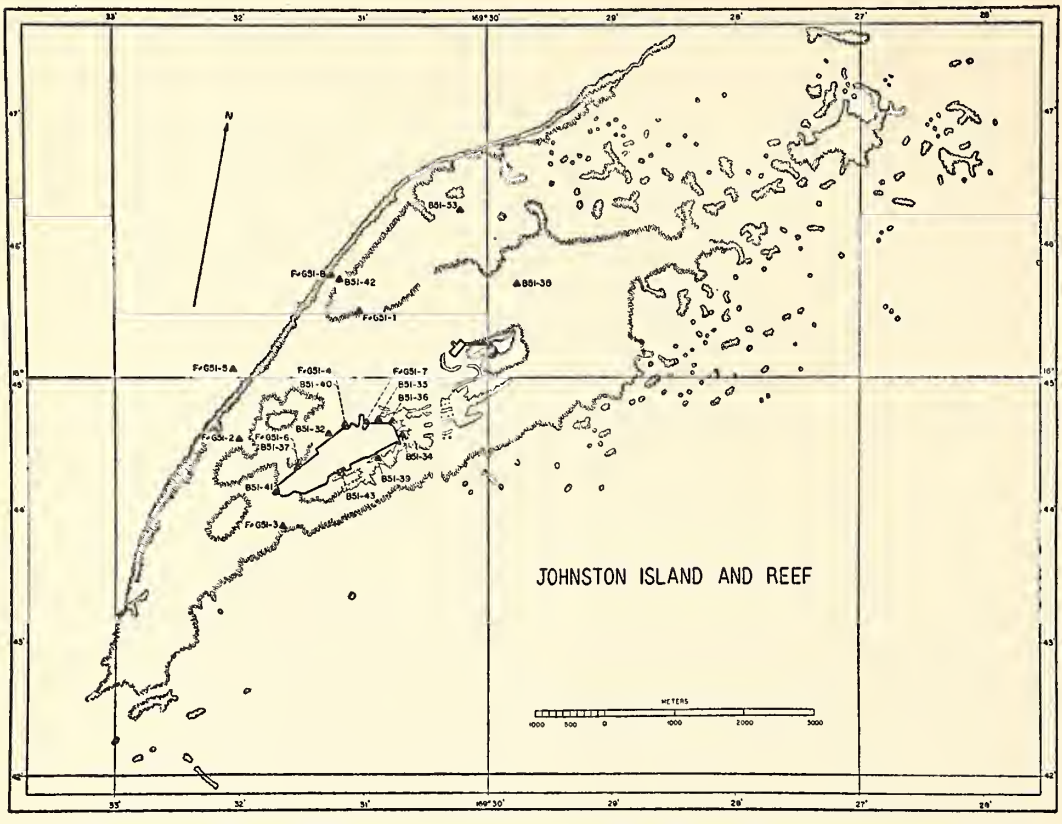

TEXT-FIG. 1. Map of Johnston Island showing field numbers of collecting stations.

of the Tanager Expedition which made a biological survey of the island in 1923. In a recent letter (1953) he stated "we caught and ate fish there, and I do not recall of anyone complaining of ill effects," and again, "I was there in 1944 and recall no mention of fish poisoning at that time."

The exact date as to when the fishes of Johnston Island became toxic is difficult to establish, but apparently it coincides with a general outbreak which appears to have started about 1943 at Palmyra, and then subsequently involved Kingman Reef, Christmas, Fanning, Midway and Johnston islands. Surprisingly, Washington and the principal Hawaiian islands apparently were not affected. Other areas may have been involved, but no record of such has come to our attention.

According to Captain John T. Martin, USAF, formerly the Base Surgeon at Johnston Island, the first documented case of fish poisoning occurred in August, 1950. Outbreaks were thought to have occurred prior to that time, but very little clinical information is available. Fishes captured inside of the lagoon were reportedly poisonous, while those taken outside of the lagoon were supposedly safe for human consumption. As a result of periodic outbreaks of fish poisoning among both civilian and military personnel it became generally known that such fishes as puffers (Tetraodontidae), triggerfish (Balistidae), saboti (*Kuhlia taeniura* (Cuvier)), ulua (*Caranx* spp.), "tuna" (probably *Euthynnus yaito* Kishinouye, a skipjack), "red or blue toothless snapper" (?), moray eels (Muraenidae), surmullet (Mullidae), etc., were dangerous to eat.

Despite the posted warnings and rigid regulations concerning the eating of reef fishes at Johnston, frequent intoxications have occurred. During the period from May, 1950, to May, 1951, it was estimated that there were approximately twenty outbreaks of ichthyosarcotoxism. In the past most of the intoxications have involved transient native civilian workers who were brought to Johnston from the Hawaiian islands. Since many of the same or similar fish species are commonly eaten in the Hawaiian area it is difficult to convince natives that the same fish elsewhere may be violently toxic.

The clinical picture of the cases that have occurred at Johnston generally resembles an acute

and often violent gastro-intestinal upset accompanied by various sensory disturbances. The onset of symptoms usually occurs within a period of two to three hours, consisting of nausea, vomiting, diarrhea and abdominal pain. The sensory disturbances, consisting of a tingling sensation in and about the mouth and extremities, may precede, accompany or develop subsequent to the gastro-intestinal symptoms. A mild to severe prostration, malaise, muscular cramps and pruritus may also be present. One patient complained of a "scalding sensation" upon defecation. Physical findings are not remarkable and treatment is generally symptomatic. There is no known antidote for the poison. Most of the patients have recovered within two or three days. Since a more complete analysis of the clinical characteristics of the disease is now in progress, no further mention will be made of the subject at this time.

Geography and Ecology of Johnston Island

Johnston Island (Text-fig. 1) is located at lat. 16° 45′ N. and long. 169° 30′ W., about 700 miles WSW of Honolulu (Hyd. Off., 1940; Robson, 1950; Bryan, 1942; Freeman, 1951). It consists of an isolated shallow bank that supports a luxuriant growth of coral-algal reefs and two small islets. The larger islet, Johnston Island, was formerly about 3,000 feet long and about 600 feet wide with a maximum elevation of about 40 feet. Sand Island, about one mile northwest of the main islet, is about one-quarter of a mile in diameter and is very low. The nearly open bank on which these islets are located is about eight miles long and is partly rimmed by an irregular, arrow-shaped, marginal coral-algal reef. The point of the arrow lies in the southwest direction. At the northeast end of the reef area are large clusters of patch reefs. Both Johnston and Sand Islands are protected from the open sea on the northwest by a ridge-like marginal reef which is five to twenty meters wide and which extends uninterrupted for about six miles in a southwest and northeast direction. A shelf-area of slightly deeper water extends out to sea for about 800 meters from the marginal reef and then drops precipitously to the 100-fathom line. The southeastern side of the bank is bordered by an expansive coral shoal area which extends for four or five miles out from the island and then slopes gradually down to the 100-fathom line and beyond. There is a dredged ship channel through the southern shoal area which makes the "lagoon" accessible to large vessels. Because of construction and dredging, the former island topography has been radically altered.

The mean tidal range is less than two feet, and the high-water interval at full and change is three hours and fifteen minutes. There is little rainfall and the island has no natural freshwater supply. During October and November, 1951, the average daily air temperature was about 30.5° C. Prevailing winds were from the east and northeast. The winds, which varied from a moderate to a strong breeze during most of the field study, made collecting very difficult.

Vegetation is very sparse, being confined to bunch grass and some small shrubs. A few coconut palms and larger shrubs have been introduced. Wild terrestrial life is limited largely to oceanic birds of the usual atoll variety. There is a general paucity of the larger forms of marine plant life. Most of the dead patch reefs, boulders, wood and metal pilings are covered with a fine growth of minute algal forms.

Reef Biochore and Collecting Stations[2]

The reef biochore of Johnston Island, for the purposes of this discussion, may be divided into the following biotopes:

Northern (Peripheral) Reef Area.—The exposed portion of the reef is comprised largely of dead corals. Living corals are located at sporadic intervals along the reef but are most plentiful along the slopes. Algal growth is minimal and consists largely of microscopic forms. The water in this general area is very clear. Water temperature on February 24, 1951, at 1400 was 25.5° C. Reef fishes are abundant in both number and species.

A. Open-water.—The seaward side of the reef is a typical open-water habitat without the protection of a coral reef. The bottom drops off precipitously. Inhabiting these offshore waters are scombroid fishes, barracuda, sharks and other pelagic species. Field No.: F&G51-5.

B. Coral.—The lagoon side of the peripheral reef and the vicinity immediately south of it are composed of corals in various stages of growth and decay. Some of the most luxuriant coral growths at Johnston are to be found in this vicinity. The water depth along the southern slope of the rim reef seldom exceeds 15 feet. Algal growth, exclusive of corallines, is minimal, and consists largely of microscopic forms. The water in this area is clear. Moray eels, butterfly, squirrel, surgeon, damsel, surmullet, puffer and file fishes are common. Eagle-rays may be occasionally observed in this biotope. Field Nos.: F&G 51-8; B51-43.

Southern (Shoal) Reef Area.—The southern reef curves around and joins the northern one at

[2] In the discussion concerned with reef ecology we have adopted the terminology as proposed by Cloud (1952).

the southwestern tip of the atoll. The reef extends northward for approximately six miles and then fades out as a series of patch reefs. This reef is comprised largely of dead coral which rests rather low in the water. Algal growth, exclusive of corallines, is minimal, consisting primarily of microscopic species. The water in this area is relatively murky compared to that of the northern reef. Water temperature on February 25, 1951, at 1400 was 25.8° C. Fish species are fewer in number and variety, compared to the northern reef.

A. Open-water.—An extensive shoal area extends south and southeast from the southern reef for about four or five miles and then gradually slopes into the deep open-water area. The water depth in most of the shoal area ranges from about seven to fourteen fathoms. Unfortunately facilities did not permit investigaton of this zone. Strong currents and a large shark population are said to exist in this area.

B. Coral.—The "lagoon" side of the southern reef consists largely of dead coral patches, knolls and rubble with scattered areas of sand. The water in this zone is generally shallow, a fathom or less in depth, and is relatively murky. A sparse fish population made this area undesirable for collecting purposes. Field No.: F&G51-3.

Bank Shoals.—The so-called "lagoon" is really the shoal water area above a partially rimmed bank that approaches but does not attain the form and structure of an atoll. It is about eight miles long and about two and a quarter miles wide at the widest point, is roughly triangular in outline, shallow and studded with numerous patch reefs of various sizes and shapes. The bank floor between patch reefs consists largely of calcium carbonate, sand and rubble of organic derivation. Reefs are most extensive in the immediate vicinity of the island. At the northwest end of the bank the reefs are broken up into a series of patchy areas which range in size from a few feet to a mile in diameter. Because of dredging, blasting and the resulting silt and debris in the water, most of the coral in the vicinity of the island has been killed. The fish population in the forementioned areas is generally sparse. However, many of the reef areas along the northern rim reef and at the northwest end of the bank support a thriving fish fauna. The water about the island, and especially on the southern side, is relatively murky, but is less turbid along the northern reef and at the northwest end of the atoll. The water temperature on February 23, 1951 at 1405 was 25.7° C., taken at station F&G51-1.

A. Patch Reefs.—The largest fish populations are generally found in the vicinity of living corals. Dead coral areas provide relatively poor collecting. Small coral knolls and other patch reefs provide an excellent habitat for many of the smaller reef fishes. Moray eels are especially plentiful in these regions. Other common inhabitants of the patch reef biotope consist of wrasse, parrot, cornet, surgeon, butterfly, squirrel, damsel and puffer fishes. Field Nos.: F&G 51-1; F&G 51-2; B51-33.

B. Sandy Areas Interspersed with Patch Reefs and Boulders.—The island shore is littered with large coral boulders which have been dredged for fill purposes. The only sandy beaches are located along the shore near stations B51-32, B51-37 and F&G51-6. The lagoon bottom in the vicinity of the island is largely of sand and patches of low, worn, dead corals. The bottom at station B51-38 is a dredged area consisting of sand and coral rubble. Station B51-38 is the garbage dumping area. The fish population is usually sparse except when refuse is being dumped, at which time sharks are plentiful. Fishes are generally most numerous in sandy areas adjoining living patch reefs. Representative fish species of this habitat are flounders, blennies, surmullet, pompano, puffers, pomacentrids, stingrays and triggerfishes. Field Nos.: F&G51-4; F&G51-6; F&G51-7; B51-32; B51-34; B51-35; B51-36; B51-37; B51-38; B51-39; B51-40; B51-43.

C. Wreckage.—The southwest end of the island is used as a dump and the shoreline is littered with scrap metal. The water attains a depth of about 12 feet in this area and is very murky. The bottom consists of coral boulders, sand, rubble and wreckage. Although this cannot be considered a natural biotope, the problem of fishes feeding around wreckage and thereby becoming toxic is one of the possibilities that must be taken into consideration. Hence, this zone is listed as a separate habitat. Species captured in this area are trumpet, parrot, damsel, pompano, trigger and butterfly fishes. Field No.: B51-41.

Taxonomic Studies

Effort was directed toward obtaining accurate taxonomic diagnoses of all material brought into the laboratory. A comprehensive systematic treatise of the fish fauna of Johnston Island is not to be found in the literature. Probably the earliest faunal study of this area is that by Smith & Swain (1882). A more recent account has been published by Fowler & Ball (1925). While the latter work includes a larger number of species, both reports are woefully inadequate. Systematic works found to be particularly useful have been published by the following authors: Schultz (1943), Weber & de Beaufort (1911-1951), Jordan & Evermann (1905), Fowler (1928, 1931, 1933, 1934, 1941), Fowler & Bean (1928, 1929, 1930), Günther (1873-1910),

Bleeker (1862-1877) and Smith (1950). In dealing with plectognaths, the nomenclature proposed by Fraser-Brunner (1935, 1941, 1943) has been largely adopted. Clark's (1949) review of the plectognath fishes was also found of value.

Dr. Leonard P. Schultz of the United States National Museum kindly supplied us with taxonomic keys from his Marshall Island report (Schultz, 1953). The authors are further indebted to Dr. Schultz for an identified collection of 743 specimens from the Marshall Islands, which proved to be of great value in making comparative studies. All of the scarids were identified by Dr. Schultz.

Representative specimens of all positive and negative material have been catalogued and preserved in the museum of the Department of Ichthyology and Herpetology of the School of Tropical and Preventive Medicine.

Methods Used in Screening Fishes

The reader is referred to the authors' (1953) report on the poisonous fishes of the Phoenix Islands for a résumé of the screening techniques of earlier workers. The technique described in the following paragraph has been adopted as the routine screening procedure for this laboratory. This method is a modification of one that was originally suggested by Karl F. Meyer and Hermann Sommer of the University of California.

Samples of the muscle, liver, intestine and gonads, whenever possible, are removed from each specimen to be tested. Care is taken to remove the sample from the right side of the fish so that the left side of the specimen remains intact for taxonomic purposes. If the fish is small it may be necessary to remove the entire viscera as a single sample. In rare instances it has been necessary to utilize the entire fish in order to obtain sufficient material for extraction. An attempt is made to remove about 7 gm of flesh for each sample. Two ml of distilled water are added for each gram of flesh. The material is then ground in a mortar or homogenized in a Waring Blendor. The homogenate is centrifuged at 2,000 rpm. for 25 minutes, using a head with an eight-inch radius. One ml of the supernatant fluid is injected intraperitoneally into each of four white laboratory mice (California Caviary Strain No. 1) weighing 15 to 25 gm. Their reactions are observed and recorded over a period of 36 hours.

Terminology Used Concerning Degrees of Toxicity.—The problem of determining the toxicity of reef fishes has necessitated developing a screening procedure which is roughly quantitative and at the same time suitable for processing large quantities of fishes within a reasonable period of time. No attempt is made to determine the LD_{50} as a routine measure because of the enormous expense involved. The classification presented in the following paragraph is an arbitrary one which does give some idea as to the degree of toxicity of a fish species within a particular geographical area. This method makes no attempt to differentiate between virulence and concentration of the toxin. The classification is based upon time in relationship to symptomatology.

Negative.—The test is negative if the mouse continues to remain asymptomatic during the maximum test period of 36 hours.

Weakly Positive.—The test is weakly positive if the mouse shows definite symptoms such as lacrimation, diarrhea, ruffling of the hair, hypoactivity, ataxia, etc., but the *animal recovers*[3].

Moderately Positive.—This term is used if the mouse develops hypoactivity, ruffling of the hair, lacrimation, diarrhea, paralysis, etc., *and dies within a period of one to 36 hours.* (It will be noted that this definition has been slightly modified from that in the Phoenix Island report (1953).

Strongly Positive.—The test is strongly positive if the mouse develops hypoactivity, ataxia and paralysis which is usually followed by clonic or tonic convulsions of varying degrees, paradoxical respiration, respiratory paralysis and *death occurs within a few seconds to one hour.*

Discussion

This study reveals that approximately 47% of the reef fishes tested from Johnson Island were toxic. The toxicity of any given species was extremely variable. The factors governing the toxicity of fishes are not understood at the present time. Apparently any species may become toxic if the proper environmental conditions are present. The spotty geographical distribution and the extreme fluctuation in toxicity within a single species indicate that the problem is basically concerned with the feeding habits of the fish. The reader is referred to the Phoenix Island report (Halstead & Bunker, 1953) for further discussion of the food chain theory.

Since many of the data in this report are of a preliminary nature, premature conclusions

[3] The "intraperitoneal injection syndrome" should be distinguished from the toxic symptoms resulting from the injection of ichthyosarcotoxins. The injection syndrome may be observed when 1 ml of distilled water is used. Immediately following the injection, respiration rate and usually depth are increased, and the animal remains normally crouched for a few minutes. This is followed by alternate stretching of the hind legs and restlessness. In some mice there is an opisthotonoid arching of the back lasting less than one minute, and which may be concomitant with any other responses.

TABLE 1. AN ANALYSIS OF JOHNSTON ISLAND FISHES WITH REFERENCE TO THEIR TOXICITY

Extract No.	Family and Species with their English and Hawaiian names	Locality No.	Part of Fish*	Results†
	ACANTHURIDAE—Surgeonfish, Tang, Maiii, Pararo			
242-1	*Acanthurus achilles* Shaw	F&G51-2	M	N
905-1,5	" "	B51-42	M	N
			V	M
502-1,3,4	" "	"	M,G,I	N
503-1,3,4	" "	"	M,G,I	N
863-1,5	*Acanthurus elongatus* (Lacépède)	B51-32	M,V	W
482-1,2	" "	"	M,L	N
483-1,5	" "	"	M,V	N
861-1,5	" "	B51-34	M,V	W
864-1,5	" "	"	M	N
			V	M
865-1,5	" "	"	M	N
			V	W
862-1,5	" "	"	M	W
			V	M
461-1,5	" "	"	M,V	N
462-1,5	" "	"	M,V	N
860-1,5	" "	B51-42	M,V	W
946-1,5	" "	"	M	W
			V	M
954-1,5	" "	"	M,V	W
216-1	" "	F&G51-3	M	N
481-1,2,4	*Acanthurus olivaceus* Bloch	B51-32	M,L	N
			I	M
520-1,5	" "	B51-34	M,V	N
899-1,5	" "	"	M	M
			V	W
539-1,2,4	" "	"	M,L,I	N
454-1	" "	B51-34	M	N
455-1	" "	"	M	N
932-1,5	*Acanthurus triostegus* (Linnaeus)	"	M	W
			V	M
922-1,5	" "	"	M	W
			V	M
463-1,5	" "	"	M,V	N
218-1	" "	F&G51-3	M	N
239-1	" "	F&G51-6	M	N
884-1,5	*Ctenochaetus striatus* (Quoy & Gaimard)	B51-34	M	N
			V	W
508-1,5	" "	B51-42	M	N
			V	W
882-1,5	" "	"	M	W
			V	N
517-1,5	" "	"	M,V	N
507-1,5	" "	"	M	N
			V	M
883-1,5	" "	"	M	W
			V	M
362-1,5	" "	F&G51-8	M,V	N

* Letters in this column refer to: M—Muscle; V—Viscera; G—Gonads; I—Intestine; L—Liver; WF—Whole Fish.

† Letters in this column refer to: N—Negative; W—Weakly Positive; M—Moderately Positive; S—Strongly Positive.

TABLE 1. *Continued*

Extract No.	Family and Species with their English and Hawaiian names	Locality No.	Part of Fish*	Results†
479-1	*Naso lituratus* (Bloch)	B51-32	M	N
480-1	" "	"	M	N
855-1,5	" "	"	M,V	W
856-1,5	" "	B51-34	M,V	W
857-1,5	" "	"	M,V	W
498-1,2,4	" "	B51-42	M,L,I	N
213-1	" "	F&G51-3	M	N
952-8	*Zebrasoma flavescens* (Bennett)	B51-42	WF	W
	ALUTERIDAE—Filefish or Leather Jacket, Oili or Ohua			
473-1,2	*Amanses carolae* (Jordan & McGregor)	B51-42	M,L	N
472-1,2	" "	"	M,L	N
943-8	*Amanses sandwichiensis* (Quoy & Gaimard)	"	WF	W
	AULOSTOMIDAE—Trumpetfish, Nunu			
937-1	*Aulostomus chinensis* (Linnaeus)	B51-32	M	W
925-1,2,3,4	" "	B51-34	M,I	N
			L	M
			G	W
924-1	" "	"	M	N
535-1,2,3,4	" "	"	M	W
			L	M
			G,I	N
550-1,5	" "	B51-41	M,V	N
	BALISTIDAE—Triggerfish, Humuhumu			
880-1,2,4	*Melichthys ringens* (Osbeck)	B51-33	M,L	W
			I	M
540-1,2,4	" "	B51-41	M,L,I	N
541-1,2,4	" "	"	M,L,I	N
542-1,2,4	" "	"	M,L	N
			I	M
477-1,5	" "	B51-42	M,V	N
499-1,2	" "	"	M,L	N
501-1,2	" "	"	M,L	N
881-1,5	" "	"	M	W
			V	M
243-1	" "	F&G51-2	M	N
211-1	" "	F&G51-3	M	W
361-1,2,4	" "	F&G51-8	M,L,I	N
521-1,2,3,4	*Melichthys vidua* (Solander)	B51-34	M,G,I	N
			L	M
492-1,5	" "	B51-42	M	N
			V	M
459-1,2,3	*Rhinecanthus aculeatus* (Linnaeus)	B51-34	M,L,G	N
845-1,2,4	" "	B51-38	M,L,I	M
543-1,2,4	" "	B51-41	M,L,I	N
	BELONIDAE—Needlefish or Saltwater Gars, Aha aha			
923-1,5	*Belone platyura* Bennett	B51-42	M	N
			V	W

* Letters in this column refer to: M—Muscle; V—Viscera; G—Gonads; I—Intestine; L—Liver; WF—Whole Fish.

† Letters in this column refer to: N—Negative; W—Weakly Positive; M—Moderately Positive; S—Strongly Positive.

TABLE 1. *Continued*

Extract No.	Family and Species with their English and Hawaiian names	Locality No.	Part of Fish*	Results†
	BOTHIDAE—Flatfish or Flounders, Pakii			
490-1,5	*Bothus mancus* (Broussonet)	B51-34	M,V	N
470-1,2,4	" "	B51-37	M,L,I	N
	CANTHIGASTERIDAE—Sharp-nosed puffers			
938-8	*Canthigaster jactator* (Jenkins)	B51-32	WF	W
	CARANGIDAE—Pompano, Ulua (large specimens) or Papio (small specimens)			
850-1,5	*Carangoides ferdau jordani* Nichols	B51-42	M,V	M
237-1	" " "	F&G51-1	M	N
230-1	" " "	"	M	N
232-1	" " "	"	M	N
233-1	" " "	"	M	N
231-1	*Caranx lugubris* Poey	F&G51-1	M	N
548-1	" "	B51-41	M	N
965-1,5	" "	B51-38	M,V	W
966-1,5	" "	"	M,V	W
468-1	" "	B51-34	M	N
468-1	*Caranx melampygus* Cuvier	B51-34	M	N
548-1	" "	B51-41	M	N
965-1,5	" "	B51-38	M,V	W
966-1,5	" "	B51-38	M,V	W
	CHAETODONTIDAE—Butterflyfish, Kikakapu or Lauhau			
547-1,5	*Chaetodon auriga* Forskål	B51-41	M	N
			V	M
500-1,2	" "	B51-42	M,L	N
505-1,5	" "	"	M	N
			V	M
478-1,5	" "	"	M,V	N
514-1,5	" "	"	M,V	N
506-1,5	" "	"	M,V	M
209-1	" "	F&G51-3	M	N
944-8	*Chaetodon citrinellus* Cuvier	B51-34	WF	M
927-1,5	*Chaetodon ephippium* Cuvier	"	M,V	W
934-1,5	" "	"	M,V	W
456-1,5	*Chaetodon ornatissimus* Cuvier & Valenciennes	"	M,V	N
524-1,5	" "	"	M,V	N
525-1,5	" "	"	M,V	N
926-1,5	" "	"	M	W
			V	M
532-1,3,4	" "	"	M,G,I	N
928-1,5	*Chaetodon punctato-fasciatus* Cuvier	B51-42	M,V	M
931-1,5	" " "	"	M,V	W
512-1,5	*Megaprotodon strigangulus* (Gmelin)	"	M,V	N
	HOLOCENTRIDAE—Squirrelfish, U'u			
956-5	*Holocentrus lacteoguttatus* Cuvier	B51-42	V	W
962-1	" "	"	M	W

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TABLE 1. *Continued*

Extract No.	Family and Species with their English and Hawaiian names	Locality No.	Part of Fish*	Results†
529-1,5	*Holocentrus sammara* (Forskål)	B51-34	M,V	N
854-1,5	*Holocentrus spinifer* (Forskål)	B51-32	M,V	W
523-1,2,4	" "	B51-34	M,L,I	N
530-1,2,4	" "	"	M,L,I	N
875-1,5	" "	B51-35	M,V	N
853-1,3,4	" "	B51-42	M,G	N
			I	W
219-1	" "	F&G51-3	M	N
236-1	" "	F&G51-4	M	N
942-8	*Holocentrus tiere* Cuvier & Valenciennes	B51-42	WF	N
504-1,5	" "	"	M,V	N
515-1,5	" "	"	M,V	N
314-1,5	*Myripristis argyromus* Jordan & Evermann	F&G51-8	M,V	N
953-8	" "	B51-42	WF	W
901-1,5	" "	"	M,V	W
900-1,5	*Myripristis berndti* Jordan & Evermann	"	M,V	W
	KYPHOSIDAE—Rudderfish, Nanue			
491-1,5	*Kyphosus bigibbus* Lacépède	B51-42	M,V	N
	LABRIDAE—Wrasse, Hinalea			
465-1,2,4 -	*Cheilinus rhodochrous* Günther	B51-33	M,L,I	N
466-1,2,4	" "	"	M,L,I	N
467-1,5	" "	"	M,V	N
963-1,5	" "	B51-42	M	N
			V	W
234-1	" "	F&G51-5	M	N
531-1,2	*Epibulus insidiator* (Pallas)	B51-34	M,L	N
846-1,5	" "	B51-42	M,V	W
847-1,2,4	" "	"	M	N
			L,I	M
215-1	" "	F&G51-3	M	N
476-1	*Thalassoma duperrey* (Quoy & Gaimard)	B51-42	M	N
	MULLIDAE—Surmullet, Goatfish, Weke or Moano			
939-1	*Mulloidichthys auriflamma* (Forskål)	B51-42	M	W
311-1	" "	F&G51-7	M	N
458-1,5	*Mulloidichthys samoensis* (Günther)	B51-34	M,V	N
935-1,5	" "	"	M	W
			V	M
496-1,5	" "	B51-42	M,V	N
902-1,5	" "	"	M,V	W
238-1	" "	F&G51-2	M	N
210-1	" "	F&G51-3	M	N
360-1,5	" "	F&G51-8	M,V	N
903-1,5	*Parupeneus bifasciatus* (Lacépède)	B51-34	M,V	W
528-1	" "	"	M	N
513-1	" "	B51-42	M	N

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TABLE 1. *Continued*

Extract No.	Family and Species with their English and Hawaiian names	Locality No.	Part of Fish*	Results†
851-1,2,4	*Parupeneus chryserydros* (Lacépède)	B51-32	M	N
			L	M
			I	W
852-1,5	" "	B51-42	M,V	W
214-1	*Parupeneus crassilabris* (Valenciennes)	F&G51-3	M	N
898-1,5	*Parupeneus trifasciatus* (Lacépède)	B51-34	M	N
			V	W
509-1,5	" "	B51-42	M	W
			V	M
904-1,5	" "	"	M	W
			V	W
212-1	" "	F&G51-3	M	N
	MURAENIDAE—Moray eel, Puhi			
464-1,5	*Gymnothorax buroensis* (Bleeker)	B51-34	M,V	N
534-1,5	" "	"	M,V	N
947-5	" "	B51-42	V	W
475-1,5	" "	"	M,V	N
217-1	" "	F&G51-3	M	N
951-1,2,4	*Gymnothorax javanicus* (Bleeker)	B51-34	M,I	W
			L	M
471-1,2,4	" "	B51-37	M,L,I	N
518-1,5	*Gymnothorax meleagris* (Shaw & Nodder)	B51-42	M,V	N
474-1,2,4	" "	"	M,I	N
			L	M
	MYLIOBATIDAE—Eagleray, Hihimanu			
948-1,2,4	*Aëtobatus narinari* (Euphrasen)	B51-42	M	N
			L,I	W
	OSTRACIONTIDAE—Trunkfish, Moa			
936-8	*Kentrocapros hexagonus* (Thunberg)	B51-34	WF	N
873-1,5	*Ctenochaetus striatus* (Quoy & Gaimard)	"	M	N
			V	W
871-1,5	" "	"	M,V	M
866-1,5	" "	"	M,V	M
867-1,5	" "	"	M	W
			V	M
868-1,5	" "	"	M	W
			V	M
869-1,5	" "	"	M	N
			V	M
870-1,5	" "	"	M,V	M
872-1,5	" "	"	M	W
			V	M
874-1,5	" "	B51-37	M,V	W
879-1,5	*Ostracion cubicus* Linnaeus	B51-42	M,V	M
	POMACENTRIDAE—Damselfish, Mamamo			
930-1,5	*Abudefduf johnstonianus* (Fowler & Ball)	B51-42	M,V	W

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† Letters in this column refer to: N—Negative; W—Weakly Positive; M—Moderately Positive; S—Strongly Positive.

TABLE 1. *Continued*

Extract No.	Family and Species with their English and Hawaiian names	Locality No.	Part of Fish*	Results†
949-1,5	*Abudefduf sordidus* (Forskål)	B51-41	M,V	W
544-1,5	" "	"	M	N
			V	W
546-1,5	" "	"	M,V	N
876-1,5	" "	"	M	M
			V	W
878-1,5	" "	"	M,V	W
545-1,5	" "	"	M	N
			V	W
877-1,5	" "	"	M,V	W
363-1,5	*Dascyllus marginatus* (Rüppell)	F&G51-8	M,V	N
941-8	" "	B51-42	WF	W
945-8	" "	"	WF	W
929-1,5	" "	"	M	W
			V	M
	PRIACANTHIDAE—Big Eye, Alalauwa or Aweoweo			
940-8	*Priacanthus cruentatus* (Lacépède)	B51-42	WF	W
240-1	" "	F&G51-4	M	N
	SCARIDAE—Parrotfishes, Panuhunuhu			
549-1,5	*Scarus brunneus* Jenkins	B51-41	M,V	N
484-1	*Scarus cyanogrammus* (Jordan & Seale)	B51-34	M	N
519-1	*Scarus duperrey* (Quoy & Gaimard)	B51-42	M	N
538-1	*Scarus forsteri* Valenciennes	B51-34	M	N
469-1,2	*Scarus perspicillatus* Steindachner	B51-37	M,L	N
485-1	" "	B51-34	M	N
486-1,2	" "	"	M,L	N
487-1,2	" "	"	M,L	N
488-1,2	" "	"	M,L	N
967-1,2,3,4	" "	"	M,L,G,I	W
526-1,2,4	" "	"	M,L,I	N
527-1,2,4	" "	"	M,L,I	N
533-1,2	" "	"	M	N
			L	W
536-1,2	" "	"	M,L	N
537-1,2	" "	"	M,L	N
493-1,2	" "	B51-42	M,L	N
522-1,2	*Scarus sordidus* Forskål	B51-34	M,L	N
511-1,5	" "	B51-42	M,V	N
516-1,5	" "	"	M,V	N
235-1	" "	F&G51-2	M	N
268-1,5	" "	F&G51-3	M,V	N
372-1,5	" "	"	M,V	N
377-1,5	" "	"	M,V	N
	TETRAODONTIDAE—Puffer, Globefish, Oopuhue or Maki-maki			
844-1,2,3,4	*Arothron meleagris* (Lacépède)	B51-32	M,L	W
			G,I	S
359-1,2,4	" "	F&G51-8	M	N
			L	S
			I	M
	ZANCLIDAE—Moorish Idols, Kihikihi Loula			
933-8	*Zanclus cornutus* (Linnaeus)	B51-34	WF	W

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† Letters in this column refer to: N—Negative; W—Weakly Positive; M—Moderately Positive; S—Strongly Positive.

Table 2. Showing the Relative Distribution of Poison among the Various Organs of the Fish

Species	Total No. of Specimens Tested	Muscle		Viscera		Liver		Gonads		Intestine		Whole Fish	
		No.	% Pos.	No.	% Pos.	No.	% Pos.	No.	% Pos.	No.	% Pos.	No.	% Pos.
Acanthuridae													
Acanthurus achilles Shaw	4	4	0	1	0	–	–	2	0	2	0	–	–
Acanthurus elongatus (Lacépède)	13	13	46	11	73	1	0	–	–	–	–	–	–
Acanthurus olivaceus Bloch	6	6	17	2	50	2	0	–	–	2	50	–	–
Acanthurus triostegus (Linnaeus)	5	5	40	3	67	–	–	–	–	–	–	–	–
Ctenochaetus striatus (Quoy & Gaimard)	7	7	29	7	57	–	–	–	–	–	–	–	–
Naso lituratus (Bloch)	7	7	43	3	100	1	0	–	–	1	0	–	–
Zebrasoma flavescens (Bennett)	1	–	–	–	–	–	–	–	–	–	–	1	100
Aluteridae													
Amanses carolae (Jordan & McGregor)	2	2	0	–	–	2	0	–	–	–	–	–	–
Amanses sandwichiensis (Quoy & Gaimard)	–	–	–	–	–	–	–	–	–	–	–	1	100
Aulostomidae													
Aulostomus chinensis (Linnaeus)	5	5	40	1	0	2	100	2	50	2	0	–	–
Balistidae													
Melichthys ringens (Osbeck)	11	11	27	2	50	7	14	–	–	5	40	–	–
Melichthys vidua (Solander)	2	2	0	1	100	1	100	1	0	1	0	–	–
Rhinecanthus aculeatus (Linnaeus)	3	3	33	–	–	3	33	1	0	2	50	–	–
Belonidae													
Belone platyura Bennett	1	1	0	1	100	–	–	–	–	–	–	–	–
Bothidae													
Bothus mancus (Broussonet)	2	2	0	1	0	1	0	–	–	1	0	–	–
Canthigasteridae													
Canthigaster jactator (Jenkins)	1	–	–	–	–	–	–	–	–	–	–	1	100
Carangidae													
Carangoides ferdau jordani Nichols	5	5	20	1	100	–	–	–	–	–	–	–	–
Caranx lugubris Poey	1	1	0	–	–	–	–	–	–	–	–	–	–
Caranx melampygus Cuvier	4	4	50	2	100	–	–	–	–	–	–	–	–

TABLE 2.—*Continued*

Species	Total No. of Specimens Tested	Muscle		Viscera		Liver		Gonads		Intestine		Whole Fish	
		No.	% Pos.	No.	% Pos.	No.	% Pos.	No.	% Pos.	No.	% Pos.	No.	% Pos.
CHAETODONTIDAE													
Chaetodon auriga Forskål	7	7	14	5	60	1	0	–	–	–	–	–	–
Chaetodon citrinellus Cuvier	1	–	–	–	–	–	–	–	–	–	–	1	100
Chaetodon ephippium Cuvier	2	2	100	2	100	–	–	–	–	–	–	–	–
Chaetodon ornatissimus Cuvier & Valenciennes	5	5	20	4	25	–	–	1	0	1	0	–	–
Chaetodon punctato-fasciatus Cuvier	2	2	100	2	100	–	–	–	–	–	–	–	–
Megaprotodon strigangulus	1	1	0	1	0	–	–	–	–	–	–	–	–
HOLOCENTRIDAE													
Holocentrus lacteoguttatus Cuvier	2	1	100	1	100	–	–	–	–	–	–	–	–
Holocentrus sammara (Forskål)	1	1	0	1	0	–	–	–	–	–	–	–	–
Holocentrus spinifer (Forskål)	7	7	14	2	50	2	0	1	0	1	100	–	–
Holocentrus tiere Cuvier & Valenciennes	3	2	0	2	0	–	–	–	–	–	–	1	0
Myripristis argyromus Jordan & Evermann	3	2	50	2	50	–	–	–	–	–	–	1	100
Myripristis berndti Jordan & Evermann	1	1	100	1	100	–	–	–	–	–	–	–	–
KYPHOSIDAE													
Kyphosus bigibbus Lacépède	1	1	0	1	0	–	–	–	–	–	–	–	–
LABRIDAE													
Cheilinus rhodochrous Günther	5	5	0	2	50	2	0	–	–	2	0	–	–
Epibulus insidiator (Pallas)	4	4	25	1	100	2	50	–	–	1	100	–	–
Thalassoma duperrey (Quoy & Gaimard)	1	1	0	–	–	–	–	–	–	–	–	–	–
MULLIDAE													
Mulloidichthys auriflamma (Forskål)	2	2	50	–	–	–	–	–	–	–	–	–	–
Mulloidichthys samoensis (Günther)	7	7	29	5	40	–	–	–	–	–	–	–	–
Parupeneus bifasciatus (Lacépède)	3	3	33	1	100	–	–	–	–	–	–	–	–
Parupeneus crassilabris (Valenciennes)	1	1	0	–	–	–	–	–	–	–	–	–	–
Parupeneus chryserydros (Lacépède)	2	2	50	1	100	1	100	–	–	1	100	–	–
Parupeneus trifasciatus (Lacépède)	4	4	50	3	100	–	–	–	–	–	–	–	–
MURAENIDAE													
Gymnothorax buroensis (Bleeker)	5	4	0	4	25	–	–	–	–	–	–	–	–
Gymnothorax javanicus (Bleeker)	2	2	50	–	–	2	50	–	–	2	50	–	–
Gymnothorax meleagris (Shaw & Nodder)	2	2	0	1	0	1	100	–	–	1	0	–	–

TABLE 2.—*Continued*

Species	Total No. of Specimens Tested	Muscle		Viscera		Liver		Gonads		Intestine		Whole Fish	
		No.	% Pos.	No.	% Pos.	No.	% Pos.	No.	% Pos.	No.	% Pos.	No.	% Pos.
MYLIOBATIDAE													
Aëtobatus narinari (Euphrasen)	1	1	0	—	—	1	100	—	—	1	100	—	—
OSTRACIONTIDAE													
Kentrocapros hexagonus (Thunberg)	1	—	—	—	—	—	—	—	—	—	—	1	0
Ostracion meleagris Shaw	9	9	77	9	100	—	—	—	—	—	—	—	—
Ostracion cubicus Linnaeus	1	1	100	1	100	—	—	—	—	—	—	—	—
POMACENTRIDAE													
Abudefduf johnstonianus (Fowler & Ball)	1	1	100	1	100	—	—	—	—	—	—	—	—
Abudefduf sordidus (Forskål)	7	7	57	7	86	—	—	—	—	—	—	—	—
Dascyllus marginatus (Rüppell)	4	2	50	2	50	—	—	—	—	—	—	2	100
PRIACANTHIDAE													
Priacanthus cruentatus (Lacépède)	2	1	0	—	—	—	—	—	—	—	—	1	100
SCARIDAE													
Scarus brunneus Jenkins	1	1	0	1	0	—	—	—	—	—	—	—	—
Scarus cyanogrammus (Jordan & Seale)	1	1	0	—	—	—	—	—	—	—	—	—	—
Scarus duperrey (Quoy & Gaimard)	1	1	0	—	—	—	—	—	—	—	—	—	—
Scarus forsteri Valenciennes	1	1	0	—	—	—	—	—	—	—	—	—	—
Scarus perspicillatus Steindachner	12	12	8	—	—	11	18	1	100	3	33	—	—
Scarus sordidus Forskål	7	7	0	5	0	1	0	—	—	—	—	—	—
TETRAODONTIDAE													
Arothron meleagris (Lacépède)	2	2	50	—	—	2	100	1	100	2	100	—	—
ZANCLIDAE													
Zanclus cornutus (Linneaus)	1	—	—	—	—	—	—	—	—	—	—	1	100
Summary	206	194	30	104	62	46	30	10	30	31	39	11	82

Table 3. Analysis of the Families Tested and Percentage Found Toxic

Families	Number of Species Tested	Percentage Positive
Acanthuridae	7	100
Aluteridae	2	50
Aulostomidae	1	100
Balistidae	3	100
Belonidae	1	100
Bothidae	1	0
Canthigasteridae	1	100
Carangidae	3	67
Chaetodontidae	6	83
Holocentridae	6	67
Kyphosidae	1	0
Labridae	3	67
Mullidae	6	83
Muraenidae	3	100
Myliobatidae	1	100
Ostraciontidae	3	67
Pomacentridae	3	100
Priacanthidae	1	100
Scaridae	6	17
Tetraodontidae	1	100
Zanclidae	1	100
Total	60	75%

regarding the edibility of Johnson Island reef fishes should be avoided. A larger series of specimens for each species must be collected and analyzed before any statistically valid conclusions can be reached. Moreover, there is some question as to the interpretation of "Weakly Positive" extracts in terms of human symptomatology.

In general it can be concluded that the viscera of a reef fish is more likely to be toxic than the somatic musculature. In the present series of fishes there were 153 specimens in which both the musculature and viscera (whole or in part) were tested; of these, 82 specimens were found to be poisonous. Fifty-three or 64% of the poisonous fishes tested had toxic musculature, whereas 80 specimens or 98% had toxic viscera.

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Summary

Fishes were collected at Johnston Island during the months of February, October and November, 1951. Fish poisoning at Johnston Island is believed to be of recent origin and a part of the general outbreak which started about 1943 at Palmyra Island, later involving other Line and Midway islands. The exact geographical distribution and causative factors concerning fish poisoning are not known. From May, 1950, to May, 1951, there were approximately 20 cases of ichthyosarcotoxism at Johnston.

Fishes were screened by preparing, whenever possible, aqueous extracts of the muscle, liver, intestine and gonads of each specimen. Four laboratory white mice were used for testing each extract. One ml of the extract was used for each mouse. The mice were observed for a period of 36 hours and then classified as negative, weakly, moderately or strongly positive on the basis of symptoms developed. Twenty-one families representing 60 species and a total of 206 specimens were tested. Of these,

Table 4. Summary of Tables 1, 2 and 3

	Species	Specimens	Muscle	Viscera	Liver	Gonads	Intestines	Whole Fish
Total Tested	60	206	194	104	46	10	31	11
Total Found Toxic	45	96	57	64	14	3	12	9
Percent Found Toxic	75	47	30	62	30	30	39	82

TABLE 5. DISTRIBUTION OF TOXIN IN MUSCLE AND VISCERA (As found in 82 specimens of a total of 153 specimens tested for both muscle and viscera toxicity)

	Viscera	Muscle	Viscera and Muscle
Number of Toxic Specimens	80	53	52
Percent Toxic of Total Tested Specimens (153)	52	35	34
Percent Toxic of Total Toxic Specimens (82)	98	64	63

45 species and 96 specimens, or about 75% of the species and 47% of the specimens, were found to be toxic. There were 153 specimens in which both the musculature and viscera (whole or in part) were tested. Fifty-three or 64% of the toxic fishes tested had toxic musculature whereas 80 specimens or about 98% of the toxic fishes had toxic viscera. Both musculature and viscera were toxic in 63 specimens or 39%. A more complete analysis of the toxicity distribution appears in Tables 4 and 5.

LITERATURE CITED

BLEEKER, P.

1862-77. Atlas ichthyologique des Indes Orientales Néerlandaises, publié sous les auspices du Gouvernement colonial néerlandais. 9 vols., 417 pls. (Not available to authors).

BRYAN, E. H.

1942. American Polynesia and the Hawaiian Chain. Tongg Publishing Co., Honolulu, pp. 35-37, 1 map.

1953. Personal correspondence.

CLARK, E.

1949. Notes on some Hawaiian plectognath fishes, including a key to the species. Amer. Mus. Nov., no. 1397, pp. 1-22, 9 figs.

CLOUD, P. E., JR.

1952. Facies relationships of organic reefs. Bull. Amer. Assoc. Petrol. Geol., vol. 36, no. 11, pp. 2125-2149, 4 figs.

FOWLER, H. W.

1928. The fishes of Oceania. Mem. Bernice P. Bishop Mus., vol. 10, pp. 1-540, 82 figs., 49 pls.

1931. The fishes of Oceania. Supplement 1. *Ibid.*, vol. 11, no. 5, pp. 313-381, 7 figs.

1931. The fishes of the families Pseudochromidae, Lobotidae, Pempheridae, Priacanthidae, Lutjanidae, Pomadasyidae, and Teraponidae, collected by the United States Bureau of Fisheries steamer "Albatross," chiefly in Philippine seas and adjacent waters. Bull. U. S. Nat. Mus., vol. 100, no. 11, pp. 1-388, 29 figs.

1933. The fishes of the families Banjosidae, Lethrinidae, Sparidae, Girellidae, Kyphosidae, Oplegnathidae, Gerridae, Mullidae, Emmelichthyidae, Sciaenidae, Sillaginidae, Arripidae, and Enoplosidae collected by the United States Bureau of Fisheries steamer "Albatross," chiefly in Philippine seas and adjacent waters. *Ibid.*, vol. 100, no. 12, pp. 1-465, 32 figs.

1934. The fishes of Oceania. Supplement 2. Mem. Bernice P. Bishop Mus., vol. 11, no. 6, pp. 385-466, 4 figs.

1941. The fishes of the groups Elasmobranchii, Holocephali, Isospondyli, and Ostarophysi obtained by the United States Bureau of Fisheries Steamer "Albatross" in 1907 to 1910, chiefly in the Philippine Islands and adjacent seas. Bull. U. S. Nat. Mus., vol. 100, no. 13, pp. 1-879, 30 figs.

FOWLER, H. W. & B. A. BEAN

1928. The fishes of the families Pomacentridae, Labridae, and Callyodontidae, collected by the United States Bureau of Fisheries steamer "Albatross" chiefly in Philippine seas and adjacent waters. *Ibid.*, vol. 100, no. 7, pp. 1-525, 49 pls.

1929. The fishes of the series Capriformes, Ephippiformes, and Squamipennes, collected by the United States Bureau of Fisheries steamer "Albatross" in 1907 to 1910, chiefly in the Philippine Islands and adjacent seas. *Ibid.*, vol. 100, no. 8, pp. 1-352, 25 figs.

1930. The fishes of the families Amiidae, Chandidae, Duleidae, and Serranidae, obtained by the United States Bureau of Fisheries steamer "Albatross" in 1907 to 1910, chiefly in the Philippine Islands and adjacent seas. *Ibid.*, vol. 100, no. 10, pp. 1-334, 27 figs.

FOWLER, H. W. & S. C. BALL

1925. Fishes of Hawaii, Johnston Island and Wake Island. Bull. Bernice P. Bishop Mus., vol. 26, pp. 1-31.

FRASER-BRUNNER, A.

1935. Notes on the Plectognath fishes. I. A synopsis of the genera of the family Balistidae. Ann. and Mag. Nat. Hist., ser. 10, no. 15, pp. 658-663, 1 fig.

1935. Notes on the Plectognath fishes. II. A synopsis of the genera of the family Ostraciontidae. *Ibid.*, no. 16, pp. 313-320, 1 fig.

1941. Notes on the Plectognath fishes. V. The families of Triacanthiform fishes, with a synopsis of the genera and descriptions of seven new species. *Ibid.*, ser. 11, no. 7, pp. 420-430, 4 figs.

1941. Notes on the Plectognath fishes. VI. A synopsis of the genera of the family Aluteridae, and descriptions of seven new species. *Ibid.*, no. 8, pp. 176-199, 9 figs.

1943. Notes on the Plectognath fishes. VIII. The classification of the suborder Tetraodontoidea, with a synopsis of the genera. *Ibid.*, no. 10, pp. 1-18, 4 figs.

Freeman, O. W.

1951. Geography of the Pacific. John Wiley and Sons, Inc., New York, 573 pp., 156 figs.

Günther, A.

1873. Andrew Garrett's fische der Sudsee. Jour. Mus. Godeffroy, vol. 1, no. 1, pp. 1-24, pls. 1-20.

1874. *Ibid.*, vols. 2-3, nos. 5-6, pp. 25-96, pls. 21-60.

1875. *Ibid.*, vol. 4, pp. 96-128, pls. 61-83.

1876. *Ibid.*, vol. 5, no. 11, pp. 129-169, pls. 84-100.

1877. *Ibid.*, vol. 6, no. 11, pp. 169-216, pls. 101-120.

1881. *Ibid.*, vol. 7, no. 15, pp. 217-256, pls. 121-140.

1909. *Ibid.*, vol. 8, no. 16, pp. 261-388, pls. 141-160.

1910. *Ibid.*, vol. 9, no. 17, pp. 389-519, pls. 161-180.

Halstead, B. W.

1951. Ichthyotoxism—a neglected medical problem. Med. Arts and Sci., vol. 5, no. 4, pp. 1-7, 8 figs.

1953. Some general considerations of the problem of poisonous fishes and ichthyosarcotoxism. Copeia, no. 1, pp. 31-33.

Halstead, B. W. & N. C. Bunker

1954. A survey of the poisonous fishes of the Phoenix Islands. Copeia, No. 1, pp. 1-11, 5 figs.

Hydrographic Office

1940. Sailing directions for the Pacific Islands. No. 166, vol. 2, pp. 400-413.

Jordan, D. S. & B. W. Evermann

1905. The aquatic resources of the Hawaiian Islands. Bull. U. S. Fish Comm., vol. 23, no. 1, pp. 1-574, 73 pls., 229 figs.

Jordan, D. S. & A. Seale

1906. The fishes of Samoa. Bull. U. S. Bur. Fish., vol. 25, pp. 173-455, pls. 33-53, 110 figs.

Martin, J.

1951. Personal correspondence.

Robson, R. W.

1950. The Pacific Islands year book. Pacific Publications Ltd., Sydney, 478 pp.

Schultz, L. P.

1943. Fishes of the Phoenix and Samoan Islands collected in 1939 during the expedition of the U. S. S. "Bushnell." Bull. U. S. Nat. Mus., vol. 180, pp. 1-316, 9 pls., 27 figs.

Schultz, L. P. & Collaborators

1953. Fishes of the Marshall and Marianas Islands. Bull. U. S. Nat. Mus., No. 202, vol. 1, pp. 1-660, 74 pls., 90 figs.

Smith, J. L. B.

1950. The sea fishes of Southern Africa. Central News Agency, Ltd., South Africa, 550 pp., 103 pls., 1232 figs.

Smith R. & J. Swain

1882. Notes on a collection of fishes from Johnston's Island, including descriptions of five species. Proc. U. S. Nat. Mus., vol. 5, pp. 119-143.

Weber, M. & L. F. De Beaufort

1911-51. The fishes of the Indo-Australian Archipelago. E. J. Brill, Leiden: 9 vols., 4184 pp., 978 figs.

7

Further Studies on Factors Influencing the Reactions of Tropical Shore Fishes to Shells

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(Plates I & II)

Introduction

In a study of the reactions of fishes which normally seek shelter in shells, it was found that the whole problem could be somewhat simplified by substituting simple tetrahedrons with a single opening, Breder (1950). The present contribution is an extension of that study. In the earlier work cardboard and concrete tetrahedrons were used. It was found that the two species employed, young *Pomacentrus leucostictus* Müller & Troschel and mature *Bathygobius soporator* (Cuvier & Valenciennes), accepted the concrete boxes as readily as shells, but that they would not spend the night in the cardboard boxes, although these were of identical form. It was thought that the slight flexibility of the water-soaked cardboard made these boxes an insufficiently rigid substitute for the stone-hard shells customarily used by these fishes. For purposes of the new series of experiments the same concrete tetrahedrons were used and in addition one which was made of glass. A black cardboard cover was made that would fit snugly over the glass construction. The aquariums used were the same as in the earlier series of experiments, 2′ × 1′ × 1′. They were supplied with running sea water. Like the others, these experiments were carried out at the Lerner Marine Laboratory on Bimini, Bahamas. They occupied a period between November 24 and December 10, 1953. These dates are mentioned mainly because fighting is at a minimum among such fishes at this season, with its cooler water, whereas in the warmer seasons fighting may be pursued with vigor, sometimes to the destruction of some of the participants.

The constructional details of the concrete tetrahedrons have been given by Breder (1950). Those of the glass tetrahedron, which was made as much like the concrete constructions as possible, follow. Four identical equilateral triangles were cut from a piece of double-thick window-glass with an ordinary steel-wheel glass cutter. Each triangle was given an altitude of 4 inches. A "doorway" was provided in one of them by the following procedure. First it was cut into three pieces by two cuts, parallel and to either side of an altitude. The central strip so obtained was then cut into three pieces by two cuts parallel to the base of the triangle. The middle piece, representing the "door," was removed and the remaining four pieces reassembled and cemented together with transparent Duco cement, leaving a rectangular "doorway" similar to those in the concrete shelters. The details of this may be best seen in Plate I, Figure 2, and Plate II, Figure 5. After the reassembled triangle with the "doorway" had become firm enough to handle, the four triangles were then cemented together to form a tetrahedron, all with transparent Duco cement. The bottom triangle together with the basal edges of the other three were then set in a thin layer of concrete in order to insure complete rigidity. This base cannot be seen in the photographs, as it is buried in the sand to a depth similar to the bases of the concrete tetrahedrons. Thus the floors of both the glass and the concrete shelters presented an identical condition: concrete, covered with a thin layer of sand.

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Experiments

Young *Pomacentrus* were handled in the following manner. Three fish were established in an aquarium with three similar concrete tetrahedrons. As is usual with this species, each individual appropriated one of the shelters as its

own. Two days later the glass tetrahedron was added to the aquarium. Except for the general attention these fish give to any small new object introduced into their aquarium, no special response was given to it and no evidence was seen of any apparent perception of it as a possible place to enter. This condition continued for three days, when the experiment was terminated.

Two new *Pomacentrus* were established in an aquarium with one concrete tetrahedron and the glass one. They behaved similarly to the fish in the previous experiment, the concrete "house" being quickly taken and the glass one not entered. The odd fish merely hid in a corner and when disturbed swam from one corner to another. At another time, when both fish were foraging, a slight disturbance caused them to beat a hasty retreat, both to the concrete chamber. In so doing one of the fish bumped into the glass shelter, evidently attempting to swim through it as fish will sometimes do on encountering the glass side of an aquarium. Certainly there was no recognition of it as a place of retreat.

The following day a black cardboard cover was placed over the glass tetrahedron. It was then occupied within five minutes. This situation is shown in Plate I, Figure 1. On removal of the cover the fish promptly swam into the concrete shelter. When the cover was replaced the fish returned. This performance could be repeated at will. It is to be noted that the other fish continually occupied the concrete shelter and that two of these young fish will not occupy one shell for long—and then only when there is considerable provocation.

The behavior of *Bathygobius* when confronted with the same situation is strikingly similar but with a few interesting modifications. In the first experiment with these fish, two were placed in an aquarium with only the glass tetrahadron present. Both the fish hid in corners and gave no attention whatsoever to the glass shelter. A concrete shelter was added the next day and it was immediately occupied by the evidently dominant fish, the other continuing to occupy a corner. Plate I, Figure 2, shows this condition with the shelterless fish foraging. This situation continued for four days at the end of which time sand was piled about the glass chamber to about half way up its sides. After this had been done the odd fish would enter it but only under conditions of vigorous disturbance, *i.e.* by being chased about by means of a glass rod. The results of this are shown in Plate I. Figure 3.

Two new fish were established in an identical arrangement and their behavior was like that of the previous two. On the second day the glass chamber was covered with black cardboard, after which it was promptly occupied, as is shown in Plate II, Figure 4. When the cover was removed, four days later, the fish showed behavior which had not been anticipated. Instead of merely swimming out of the construction, as had the *Pomacentrus* under identical conditions, the *Bathygobius* showed what appeared to be a state of "confusion." The fish was seen, on removal of the cover, to be adhering upside down near the apex of the tetrahedron, a position not unusual with these fishes. It continued to cling with its pelvic suction disc for a moment and then descended to the floor of the chamber where it executed a series of attempts to force its way through the now transparent walls. It worked back and forth along the two walls without an opening, in evident confusion, before finding the doorway which it formerly had been using regularly and with certainty. Once emerged from the glass shelter, it immediately and with no hesitancy entered the concrete retreat. On replacement of the cover the fish reentered the glass "house" within five minutes.

An hour later the cover was once again removed, whereupon the fish dropped to the floor and quietly and promptly left through the doorway, with no evidence of confusion, after which it entered the concrete shelter as before. A similar trial three hours later again revealed some of the earlier confusion, but not nearly so marked. The two fish were then left undisturbed for four days. At the end of this time removal of the cover produced confusion equal to that seen the first time it was tried. As in the first trial, the fish seemed to insist on finding an exit every place except through the doorway. This experiment could be repeated at will, the amount of "confusion" induced being very nearly directly proportional to the length of time the black cover had remained in place. Plate II, Figure 5, shows conditions as the cover was being removed. The cover is still partly in the water, in the upper left, and the fish is in its typical position, just a moment before it dropped to the floor of the chamber.

Discussion

As noted by Longley & Hildebrand (1941) and Breder (1950), *Pomacentrus* is strictly diurnal and spends the dark hours entirely within the shelter of its selection. While individuals may have some slight contact with the cavity they inhabit, such as with the tips of the pectoral or pelvic fins, they are not ordinarily in intimate contact with the walls or floor of their shelter. *Bathygobius,* on the other hand, is practically aperiodic in habit and does have con-

tinual intimate contact with the walls or floor of the shelter, always resting on the bottom or hanging on the walls or ceiling by means of its pelvic suction disc. For this reason it was thought that there might be some marked difference between the reactions of *Bathygobius* to a transparent shelter and those of *Pomacentrus,* on the assumption that much of the behavior in reference to shelters shown by the former is dependent on tactile cues, whereas that of the latter would seem to be largely, if not entirely, visual.

Before going into a discussion of the experimental results it is necessary to point out that in the earlier work on these species in aquaria there was noted a reluctance on the part of well-established fishes to enter a newly introduced shelter, although it would be thoroughly "inspected." It is to be especially noted that in the present experiments, in each case where the glass shelter was introduced the fish could not, in any sense, be considered as well established. They were merely given sufficient time to get well over their initial fright incident to netting and general handling before the experiments were started. These various details of behavior, together with the other items noted, especially as shown by the experiments with the black cover, indicated clearly that it is the transparency of the glass shelter which prevents its normal use by both species. The tactile cues which *Bathygobius* receives over and above the chiefly non-tactile cues received by *Pomacentrus,* are evidently insufficient to make any marked difference in the attitudes of the two species toward transparent shelters.

It is to be noted that in the earlier study it was possible to write as follows regarding the introduction of opaque objects into the aquaria: "As is obvious from the most casual observations, these experiments confirm the fact that both species under discussion are acutely aware of the physical features of their environment. They both spend much time swimming around and nosing into crevices of any new object or one which has been turned around or otherwise disturbed. As was noted by Breder (1949), they also will frequently return an object to its original site if they are capable of doing it. *Bathygobius* generally perches itself on the new object after it has 'inspected' it for a time, perhaps obtaining further sensory data through the pelvic sucker. *Pomacentrus,* on the other hand, seldom touches such objects." In none of the present experiments was the glass retreat "inspected" or touched in any exploratory sense by either species. This is the more remarkable for, as can be seen in the photographs, the glass was not perfectly clear, because of the settling of detritus, although at all times distinct vision through the glass was possible.

The confusion effect noted in the case of *Bathygobius,* but not in *Pomacentrus,* may indeed be a measure of the difference between these two fishes in respect to the differing nature of their manner of obtaining sensory cues. In the latter, clearly dominated by visual cues, there was no confusion whatever and an opening remained "a doorway" even if the surrounding walls suddenly became transparent. *Bathygobius,* on the other hand, always in contact with some solid except when actively swimming, showed confused responses. Swimming, in this species, is never long nor continuous, but is more in the nature of short hops from place to place. It is nevertheless difficult to account for the failure of these fish to recognize the normally-used doorway. Beebe (1931) noted that individuals removed from a tidepool to another usually returned to their original home pool without difficulty. Aronson (1951) in other connections concerning the tidepools which these fishes often inhabit, found them to have a normal, if not superior, fish-memory for places and the location of objects, exits and the like. The work of Goldsmith (1905, 1912, 1914) on the topographic memory of *Gobius minutus* Linnaeus gives similar data on a related species. Thus the evidence from both the present and earlier studies (Breder 1948, 1949, 1950) and from the above references reinforces the idea that these fishes have a very acute "awareness" of the micro-geographic details of their immediate environment and a considerable retention as to the nature of those details.

It is consequently believed that the confusion of gobies in glass retreats suddenly uncovered is not rooted in any inadequacy of either memory or awareness of objects and their locations. It is thought, rather, that this failure to escape promptly from a retreat which has suddenly become transparent is rooted in a breakdown of whatever integrative mechanism is involved. The failure to react appropriately to a transparent wall of glass suggests a characteristic inability to recognize a transparent solid for what it is. Ordinarily in the environment in which these fishes live, the ability to see the view ahead also means the ability to move ahead. It should be recalled that some fishes never learn to respect the glass walls of an ordinary aquarium while others do so in a very short time. Also, it may be noted, well-established gobies will often adhere to the transparent glass sides of their aquarium while newly introduced ones will do this only where the corner angles present the surface as an opaque solid. At such

times newly introduced fish will still try to swim through the sides. Eventually they seem to become accustomed to the glass walls but seemingly never to the point where a piece of food offered from the outside or a fly alighting on the glass wall will fail to elicit a feeding response.

Although bottles of various kinds are present in the sea-bottom environment of these fishes and are not infrequently occupied by either species, they are, for the most part, covered with marine growths, half buried in sand or made of brown or green glass. Experiments made with clear glass bottles in the sea indicate that while not many gobies will enter the mouths of small-mouthed bottles, when they do they have difficulties in finding their way out. This behavior, on a basis of appearance, would seem to be identical with that found in connection with the glass tetrahedron. The infrequency of entry into such bottles by either species is apparently associated with some difficulty in finding such a small opening in a transparent object.

When the opening is made more accessible we have, in effect, a fish trap. In their more common forms the glass or plastic minnow traps take advantage of this situation, as indeed do wire traps or fykes or other devices where the fish can see ahead but are not able to swim ahead. Of course, in addition, these traps are usually baited and the mouth is made easy to find from the outside but hard to find from the inside by some expression of the simple funnel principle. In the case of a common bottle the "funnel" actually points the wrong way, from a fish trapper's point of view. That a simple clear bottle or a glass tetrahedron (with which the fish was habituated) should so confuse such a fish is somewhat surprising.

All this suggests that visual stimuli and tactile stimuli are closely integrated in the case of *Bathygobius*. If this can be accepted, then the reversal of one (transparency where opaqueness would be expected) with no modification of the associated tactile stimuli might well lead to inadequate responses on the part of the fish. The two sets of stimuli are then no longer acting in the normal unison with which the individual has had experience. *Pomacentrus* has no such problem, because of the minor or absent role of contact in the totality of its responses to solids.

These experiments would seem to indicate that any thigmotropic effect operating in the case of *Bathygobius* is insufficient to override the visual cues to a point where the fish can accept a transparent shelter. Further than this, if an opaque shelter has been accepted by a fish and then is suddenly rendered transparent, whatever positive thigmotaxis may be involved is clearly insufficient to prevent immediate rejection of the shelter.

Bathygobius shows a wide variety of changeable color patterns which have been described in general terms by Beebe (1931), discussed as to some of their significance by Breder (1943, 1949) and described in much greater detail by Tavolga (1950) who included a demonstration that different populations showed differences in the range of patterns displayed. Because of these differences and the behavior herein discussed, it would appear that this species should present favorable material for a more refined study. The interaction between the acceptance of differing kinds of retreats by individuals differing in physiological and psychological condition, as indicated by the pattern shown, should be amenable to experimental approach. A comparative study of how these two kinds of behavior, pattern change and shelter entry, are modified in populations differing as to the range and scope of patterns regularly displayed, should be illuminating.

Summary

1. Young *Pomacentrus leucostictus* and mature *Bathygobius soporator* will accept small artificial concrete shelters for occupancy in aquaria.
2. The same individuals will completely disregard clear glass shelters of the same design.
3. They will accept the glass ones if they are made opaque by covering with a black paper cap.
4. Removal of such an opaque cap will cause the *Pomacentrus* to leave directly and rapidly.
5. Removal of such an opaque cap will cause *Bathygobius* to enter a state of "confusion" wherein it attempts to leave through the glass walls at practically every point except the doorway which it had been using previously, before it finally finds its way out.
6. This confusion lessens with repeated trials but returns again in force if a few days elapse between trials.
7. The confusion is evidently not rooted in either faulty memory or inadequate knowledge of the details of the immediate environment, but rather in an inability to "comprehend" the nature of a transparent solid.

8. The difference between *Pomacentrus* and *Bathygobius* in this respect is evidently based on the fact that the former receives chiefly visual cues from solids whereas the latter receives both visual and tactile cues from such objects, the failure of the two types of stimuli to properly integrate producing the observed confusion.

References

Aronson, L. R.

1951. Orientation and jumping behavior in the gobiid fish *Bathygobius soporator*. Amer. Mus. Novitates, (1486): 1-22.

Beebe, W.

1931. Notes on the gill-finned goby, *Bathygobius soporator* (Cuvier and Valenciennes). Zoologica, 12 (5): 55-66.

Breder, C. M. Jr.

1943. The eggs of *Bathygobius soporator* (Cuvier and Valenciennes) with a discussion of other non-spherical teleost eggs. Bull. Bingham Oceanogr. Coll., 8 (3): 1-49.

1948. Observations on coloration in reference to behavior in tidepool and other marine shore fishes. Bull. Amer. Mus. Nat. Hist., 92 (5): 281-312.

1949. On the relationship of social behavior to pigmentation in tropical shore fishes. *Ibid.*, 94 (2): 83-106.

1950. Factors influencing the establishment of residence in shells by tropical shore fishes. Zoologica, 35 (12): 153-158.

Goldsmith, M.

1905. Recherches sur la psychologie de quelques poissons littoraux. Bull Inst. Gén. Psychol., 5: 51-67.

1912. Contribution à l'étude de la mémoire chez les poissons. *Ibid.*, 12: 161-176.

1914. Les réactions physiologiques et psychiques des poissons. *Ibid.*, 14: 97-228.

Longley, W. H. & S. F. Hildebrand

1941. Systematic catalogue of the fishes of Tortugas, Florida with observations on color, habits, and local distribution. Papers Tortugas Lab., 34, Carnegie Inst. Washington Publ. (535): 1-331.

Tavolga, W. N.

1950. Pattern variability among populations of the gobiid fish *Bathygobius soporator*. Copeia, (3): 182-194.

EXPLANATION OF THE PLATES

PLATE I

Behavior of fishes in reference to standardized retreats.

FIG. 1. Two *Pomacentrus* occupying respectively a concrete and a covered transparent shelter.

FIG. 2. Two *Bathygobius*, one occupying a concrete shelter and the other refusing a transparent shelter.

FIG. 3. The two fish of Fig. 2 after sand has been banked about the transparent shelter.

PLATE II

Behavior of fishes in reference to standardized retreats.

FIG. 4. The two fish of Plate I, Figs. 2 and 3, after the transparent shelter has been covered.

FIG. 5. Detail of the position generally taken by the occupant of the glass shelter. This photograph was taken the moment the cover was removed and before the fish had dropped to the floor of the chamber. The cover may be seen in the upper left corner and the near corner of the concrete chamber in the lower right. Photographs by Carol Mosher.

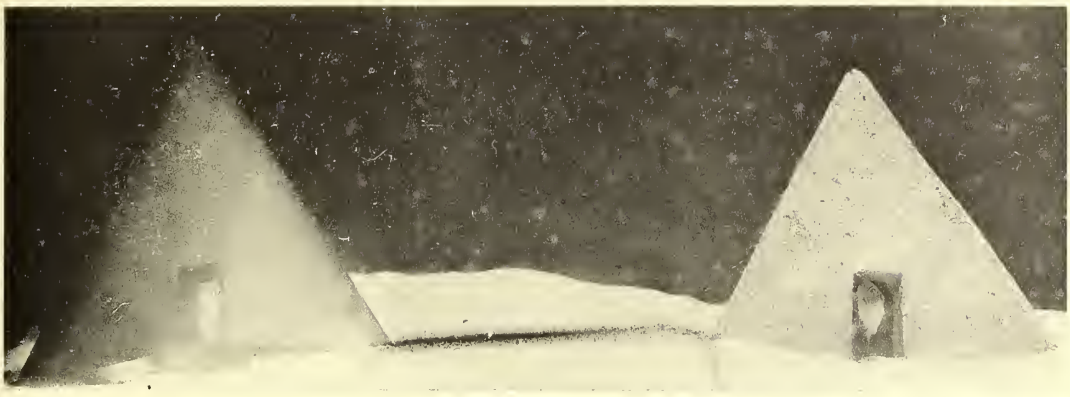

FIG. 1

FIG. 2

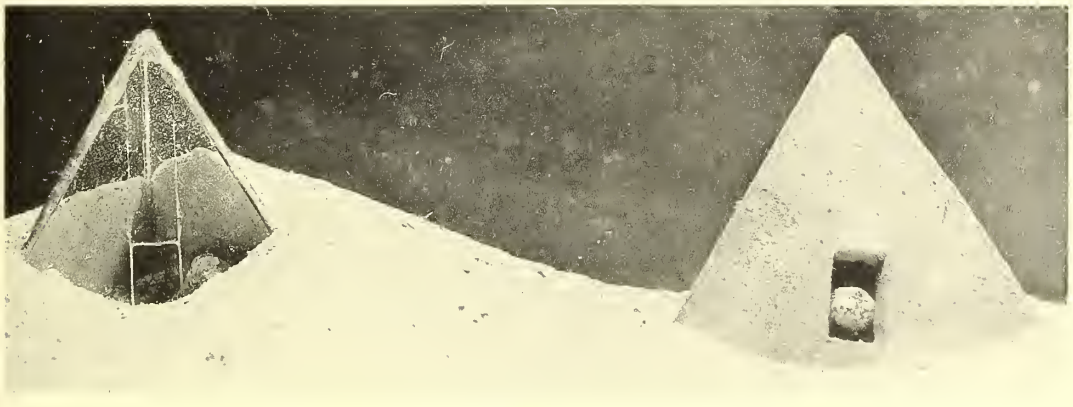

FIG. 3

FURTHER STUDIES ON FACTORS INFLUENCING THE REACTIONS
OF TROPICAL SHORE FISHES TO SHELLS

FIG. 4

FIG. 5

FURTHER STUDIES ON FACTORS INFLUENCING THE REACTIONS
OF TROPICAL SHORE FISHES TO SHELLS

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Contents

8

Spectral Reflectance Characteristics of Butterflies (Lepidoptera) from Trinidad, B.W.I.[1]

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Department of Tropical Research, New York Zoological Society, New York 60, N. Y.

(Plates I-III; Text-figures 1-9)

[This paper is one of a series emanating from the tropical Field Station of the New York Zoological Society, at Simla, Arima Valley, Trinidad, British West Indies. This station was founded in 1950 by the Zoological Society's Department of Tropical Research, under the direction of Dr. William Beebe. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of undisturbed government forest reserves. The laboratory of the station is intended for research in tropical ecology and in animal behavior. The altitude of the research area is 500 to 1,800 feet, with an annual rainfall of more than 100 inches.

For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," William Beebe. (Zoologica, 1952, Vol. 37, No. 13, pp. 157-184.)]

CONTENTS

INTRODUCTION

ALTHOUGH butterflies are to human eyes among the most colorful members of the animal kingdom, little is yet known of the physical characteristics of the colors themselves. Similarly, the possible adaptive significance of colors and patterns in intraspecific insect behavior remains largely unexplored. Because of differences in human and insect vision, a study of the role of color in the social behavior of any species of butterfly must be built on a knowledge of the spectral composition of the various colors of its wings. Since butterflies, along with the majority of insects, are visually

[1] Contribution No. 950, Department of Tropical Research, New York Zoological Society.

sensitive to the near ultraviolet, this short-wave region is also important. The present investigation of the spectral composition of certain butterfly colors, in both the visible and ultraviolet, was therefore undertaken as a prerequisite to the study of social behavior in these same species.

The colors of many butterflies depend not only on direct reflectance from pigment deposited in the scales, but also in part on the structure of the scales themselves. These physical colors, here classified as iridescent phenomena, may be caused by a variety of means—by the scattering, reflection and refraction of light from the scale surfaces, or by interference between reflections from superimposed, microscopic plates. Because of the prevalence of these non-pigmentary colors, absorption and transmission spectra of extracted pigments are not reliable bases for determining the portions of the spectrum which actually reach the insect eye.

The current problem, therefore, involved the working out of a practical method for recording the spectral composition of the colors, regardless of their origin. There were three important requirements to be met. The procedure must be able to give valid general analyses both of entire butterfly wings and of minute portions of patterns; it must be suitable for use by non-physicists working under tropical conditions with limited laboratory facilities; finally, it must also be applicable to fresh flowers and to fast-fading, non-lepidopterous insects, in connection with various other studies of behavior. The photographic method described in the following pages has met these requirements adequately.

In the present paper the term "reflectance" is used in its broadest sense, in opposition to "absorption" and "transmission," to include color phenomena caused not only by simple reflectance from pigment, but by any structural means as well.

Deep appreciation goes to Dr. Y. K. Roots of the Physics Department, New York University, who advised me on the general method, gave many most helpful suggestions and lent the use of his department's densitometer for negative analysis. Hearty thanks are also due to Mr. and Mrs. C. Reed Cary of Philadelphia for their gifts of interference filters, and to Messrs. John Duane and Daniel Smith, both of the Interchemical Corporation, New York, for providing ultraviolet spectrophotometric analyses of standards. Finally, I am particularly grateful to Dr. William Beebe, Mr. Henry Fleming and Miss Rosemary Kenedy of this Department for their help in the field and for advice in many particulars. All systematic identifications are through the kindness of Mr. Fleming.

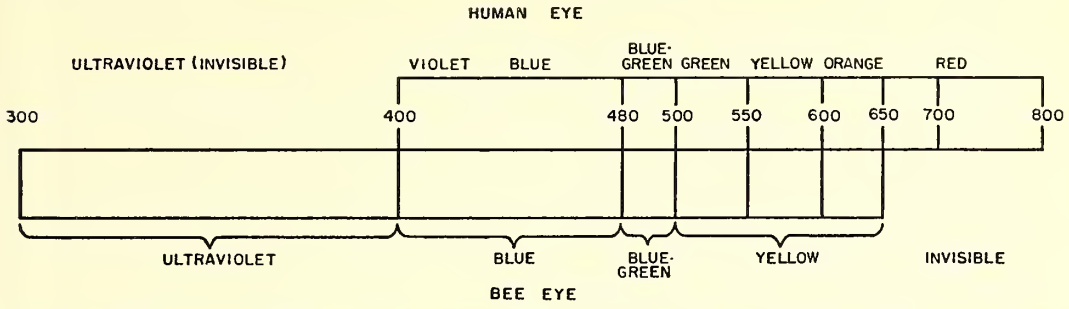
Historical Review

It appears that no one has yet published spectrophotometric reflectance curves from the natural wing surfaces of butterflies. The work most closely related has been concerned with butterfly patterns in general and with the pigments in the scales, from the points of view of their chemistry, physiological origin, development and, occasionally, of their possible application to problems of systematics. The following key references will give a survey of the subject: Cockayne (1924); Coste (1890-1891); Ford (1941-1947); Fox (1953); Hopkins (1895); Köhler (1926); Mayer (1896-1897); Mayer & Cook (1943); dos Passos (1948); Richards (1951); Schmidt (1942); Thomson (1926); Timon-David (1947); Wigglesworth (1924, 1946, 1949).

One of the first of these, Mayer (1897, p. 173), observed the colors of a few of the butterflies in his classic study by means of a small direct vision spectroscope, and noted, "In general it was found that the colors of the wings are not simple, but compound; that is to say, they are made up of a mixture of several different colors."

Some of the above references, notably Fox (1953) and Richards (1951), also discuss the physical characteristics of structural colors in butterflies and review the literature. Anderson & Richards (1942), Gentil (1942, 1943), Kühn (1946), Kühn & An (1946) and Suffert (1924) are entirely concerned with structural colors, either in butterflies alone or in insects including butterflies. The Anderson & Richards study of the interference colors of blue *Morpho* butterflies with the electron microscope is the most thorough ever made of such insect colors, and is invaluable for an understanding of the subject.

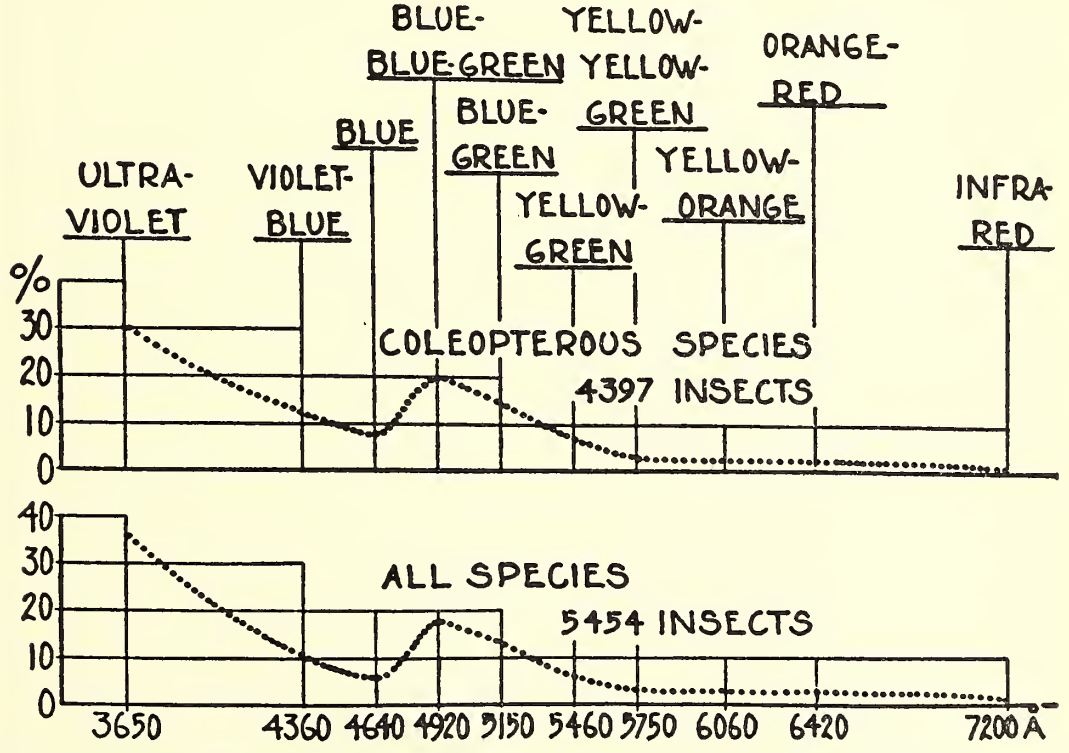
The photographic approach to analysis of butterflies' colors has apparently been used only twice—first by Lutz (1933.1) who published prints of a number of species taken through an ultraviolet filter. His purpose was to indicate how a butterfly's pattern might differ in appearance to another insect, sensitive to ultraviolet, in comparison with its appearance to human beings. However, he did not in his photographs take into account the fact that insects are also sensitive to practically all of the humanly visible spectrum, although at least most of them are only weakly sensitive to the orange and red. Brues (1941) accordingly photographed a series of butterflies not only through an ultraviolet filter alone but again for comparison, through a blue filter which both admitted some ultraviolet and cut off the orange and red. This arrangement, he considered, would thoretically give a rendition of the pattern which should approach in relative



TEXT-FIG. 1. Comparison of the visible spectrum for the human eye (above) and for the honeybee (below). (Redrawn after von Frisch, 1948, 1950). Wavelengths in millimicrons. Cf. Text-fig. 2.

values of light and dark that perceived by the insect. The high sensitivity of the negative material to ultraviolet rays, and the relatively low intensity of these rays in his particular source, would roughly compare, he suggested, with natural conditions: although the insect eye is extremely sensiitve to ultraviolet (Text-figs. 1, 2), little of this region penetrates the earth's atmosphere (Text-fig. 3). However, Brues' purpose was not to determine relative reflectance in the various spectral regions, or to any standard, and hence his discussion has no direct application to the present study.

As indicated above, the insects' sensitivity to the quality of light is inseparably connected with the problem of any adaptive evolution of their wing colors in intraspecific relations. Because of this connection, it seems desirable to give here a brief review of the current state of knowledge of this aspect of insect vision, so that the implications of the spectral analyses to be given may be more promptly clear. This is naturally divided into two sections: the first concerns the limits of the spectrum visible to insects, and the relative sensitivity of the insect to the various spectral regions. The second is concerned purely



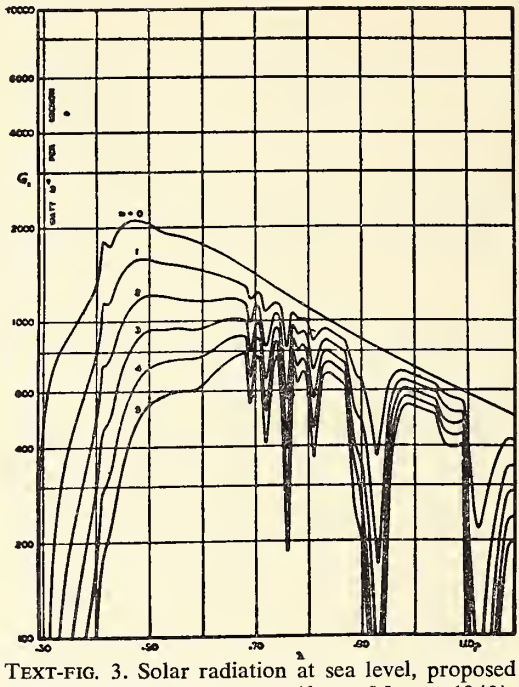
TEXT-FIG. 2. Group behavior curves of insects to colored lights. (From Weiss, 1943.) Wavelengths in Angstrom units. "All species" in the lower diagram included, in addition to the 4,397 Coleoptera shown in the upper figure, more than a thousand Diptera, Hemiptera and Hymenoptera.

with such perception or "color vision," including proof of differentiation of colors from all shades of gray, and the number of separate hues distinguished.

It has been known since the work of Sir John Lubbock (1882) that ants are not visually sensitive to the identical stretch of the electromagnetic spectrum which forms, for human beings, the visible spectrum. All insects which have since that time been adequately tested, including a number of Lepidoptera, have proved to be optically sensitive to the near-ultraviolet and many, at least, have very weak or negligible sensitivity in the orange-red and red. Weiss (1943.2, 1944, 1945, 1946) has published critical reviews of both early and recent work, with the emphasis on strictly laboratory experiments concerning the insects' responses to light of various colors. Ilse (1941) reviews work, including her own, on color vision in bees and butterflies based on observations and experiments either in the field or in insectaries, and involving the insects' behavior in seeking food, mates, etc.

For human beings in ordinary daytime vision there is a single peak of sensitivity in the yellow-green. For honey bees, *Drosophila*, many beetles and one hemipteron, tested by group behavioral responsiveness to light, it has been shown definitely that there are two peaks (Text-fig. 2). In all of these insects and probably in the vast majority, if not all, of other eyed insects, the major peak is in the near ultraviolet (Bertholf, 1931, 1932; Weiss, 1943.1; Weiss *et al.*, 1941, 1942), at least for more distant vision (Weiss *et al.*, 1941). In honey bees, according to other tests, the second, minor peak is in the yellow-green, similar to the single peak in man (Bertholf, 1931); this is not shown on Text-fig. 2. Without reference to the ultraviolet, Schlieper (1927) and Ilse (1932.1) gave evidence that between blue and red the peak of two species of nymphalid butterflies (*Vanessa*) is also in the yellow-green. In *Drosophila* and many beetles, however, the peak in the humanly visible region is in the blue-green or blue-blue-green (Bertholf, 1932; Weiss, *loc. cit.*; Weiss *et al.*, *loc. cit.*). These peaks of sensitivity do not indicate in themselves that the insects have color discrimination; it is only that when large numbers are tested, by various means, the majority respond most readily to light of these particular wavelengths, when given a choice of other bands of equal intensity. Furthermore, when intensities are increased adequately, but not excessively, positive responses may be elicited all the way up to 720 mμ (Weiss, *loc. cit.*; Weiss *et al.*, *loc. cit.*).

Electroretinograms of a grasshopper, a moth (*Samia cecropia*) and a beetle (*Dytiscus*) give



TEXT-FIG. 3. Solar radiation at sea level, proposed standard radiation curves (from Moon, 1940). Wavelengths in microns. Reproduced to show the sharp cut-off of ultraviolet (.30-.40μ) penetration to the earth with increase of air mass (m). The extent of the visible spectrum for insects is approximately .30μ to .65μ; for man, .40μ to .70μ. Cf. Text-figs. 1 and 2.

results comparable with the group behavior responses of beetles, *Drosophila* and Hemiptera cited above, the electroretinograms showing a major peak in the green (about 520 mμ); responses in the ultraviolet were not tested (Crescitelli & Jahn, 1939; Jahn & Crescitelli, 1939; Jahn & Wulff, 1948). A similar peak was shown in the electroretinograms of single visual cells of the horseshoe crab (*Limulus*) (Graham & Hartline, 1935). As Weiss sums up (1944, p. 271): "Thus it appears that both the electrical responses of the insect eye and the motor responses of the insect itself to different colors of equal intensity are due to differences in sensitivity, or to the absorption of light, which varies with wavelength, by the primary photosensitive substance of the visual sense cells, and are not the effect of wavelength by itself."

Only in honeybees has actual color discrimination in insects been thoroughly investigated. Thanks especially to the work of von Frisch (1915), Kühn (1927) and Lotmar (1933), it is well established that four separate hues are distinguished (Text-fig. 1): bee-ultraviolet (from about 300-400 mμ), bee-blue, covering the human violet and blue (400-480 or 490 mμ), bee-blue-green (480 or 490 to 500 or 510 mμ)

and bee-yellow, covering a long stretch of spectrum which, for human beings, is seen as green, yellow, orange and orange-red. (510-650 mμ). Furthermore, these four hues consist, for the bees, of two complementary pairs (Hertz, 1937.1, 1937.2, 1937.3, 1939). Just as red and blue-green, orange and blue, and yellow and violet are complementary for human beings, so is bee-ultraviolet complementary to bee-blue-green, and bee-blue to bee-yellow. Finally, because of their sensitivity to ultraviolet, "white" for a bee must include reflectance in the ultraviolet as well as throughout the visible. Many objects, including flower petals, male pierid butterfly wings and zinc oxide white pigment, all of which appear white to human beings, reflect virtually no ultraviolet and are distinct to bees, including meliponids (Lutz, 1933.2), from positively ultraviolet whites. They appear, in fact, to affect the bees in the same fashion as bee-blue-green, since the complementary components in the white, bee-blue and bee-yellow, "cancel out." A comparable situation exists for human beings where a surface strongly reflects light of all visible major hues save one—violet, for example; such a surface is seen as pale yellow; this, of course, is the complementary of the absent hue, lightened by the "cancelled out" complementaries of the remainder of the visible spectrum.

Work on color discrimination in butterflies is less complete than in honeybees, but some butterfly families have already been shown to have well developed color discrimination, which in some cases appears to be similar to that of bees. Ilse (1928), working with European butterflies, found that nymphalids distinguish at least three hues in the visible region—a "blue," "green" and a "yellow" which, as in bees, apparently does not extend much beyond the orange; the ultraviolet region was not included in this study. In addition, her experiments suggested strongly that in pierids and papilionids the spectrum is extended well into the red, though red is not necessarily for them a distinct hue; she also showed (1937) that for the cabbage butterfly (Pieridae) at least, violet, mauve or reddish is complementary to a blue-green-to-green hue, which would seem to exclude, for pierids, the possible complementariness of this green to ultraviolet.

Preliminary work of our own, not yet published, on butterflies in Trinidad proves that butterflies in general undoubtedly have high sensitivity in the ultraviolet, and that these butterflies include both pierids and papilionids, as well as nymphalids, morphids, heliconiids, ithomiids and danaids. Further, we corroborate Ilse's findings that butterflies decidely distinguish all hues, including non-ultraviolet white, from all shades of positively-ultraviolet gray. Work on spectrum extension into the red, the numbers of hues discriminated and their system of complementaries is still proceeding.

Using North American wildflowers as subjects, Lutz and Richtmyer inaugurated work similar to our own in their investigation of ultraviolet reflectance from flowers. Richtmyer (1923) used a small, portable quartz spectrograph, which was operated directly in the field. In a correlated study Lutz (1924) used ultraviolet, blue and red filters, combined with pinhole photography, for roughly determining spectral reflectance in the three regions. In both studies, suitable use was made of magnesium oxide standards for comparison. At that time the fact that bee-ultraviolet and bee-blue-green are complementary colors was not known, and hence the particular importance of the blue-green region. However, as it turned out, only four species out of 25 tested proved to reflect any possibly significant proportion of ultraviolet; these consisted of three yellow flowers and one rose-purple species. In 1933 Lotmar investigated European flower colors spectrographically in the laboratory, agreeing with Lutz's results in recording very low to negative ultraviolet reflectance in the vast majority of tested flowers. In Trinidad our own spectral examinations of tropical flowers have given similar results (unpubl.).

Method

All of the background summarized in the preceding section was borne in mind in working out the method used in this study. Some details are included which may seem over-obvious to physicists, and others which will appear equally uncalled for to a practicing zoologist. However, it is increasingly clear that when one profession tackles the techniques or materials of the other, the most elementary details are the ones which often cause the most time-consuming delays and preliminary mistakes.

The requirements of the method may be divided as follows:

1. It must be useful for color analyses of insects, flowers and leaves in general.

2. It would be necessary to determine spectral reflectance from the near ultraviolet to the red, covering the ultraviolet penetration of sunlight to the earth. Since both the penetration of sunlight and the transmission of bee ommatidia fall off sharply below 366 mμ, that figure was taken as an adequate lower limit for the required spectra. The shortest rays which reach the earth measure only 290 mμ; however, these are present in sunlight only under ideal weather conditions (Text-fig. 3). It was not necessary to secure measurements of exact reflectance at individual wavelengths, or at extremely narrow bands of

TABLE 1. FILTER COMBINATIONS USED IN SPECTRAL REFLECTANCE STUDY
(cf. Text-fig. 4)

Filters	Light Source	Color for Man	Approx. Transmission Range ($m\mu$) (under conditions used)	Peak ($m\mu$)	Remarks
Wratten 18 A	Sun	Black (UV)	290–400	366	
Wratten 18 A	"Mineral-light" SL 3660	Black (UV)	290–400	366	Peak of source extremely narrow
Wratten 18 A + Bausch & Lomb Interference KD 4002	Sun	Black (UV)	290–400	380	
Wratten 35(D) + 47(C5) + 2 A	GE Photoflood 2 A	Violet-blue	400–475	430	
Wratten 35(D) + 45(H)	GE Photoflood 2 A	Blue	430–475	450	
Wratten 75(n)	GE Photoflood 2 A	Blue blue-green	475–508	490	
Wratten 58(B2) + 45(H)	GE Photoflood 2 A	Blue-green	475–545	510	
Wratten 15(G) + 45(H)	GE Photoflood 2 A	Green	508–548	525	
Wratten 58(B2) + 15(G)	GE Photoflood 2 A	Green-yellow	506–615	540	
Wratten 58(B2) + 22(E2)	GE Photoflood 2 A	Yellow	548–618	570	
Wratten 25(A) + Bausch & Lomb Interference 600 $m\mu$	GE Photoflood 2 A	Orange	590–660	600	
Wratten 29(F)	GE Photoflood 2 A	Orange-red	600–650	640–660	Used in preliminary work only
Wratten 25(A) + Bausch & Lomb Interference 650 $m\mu$	GE Photoflood 2 A	Orange-red	590–660	650	

wavelengths; only the *relative* reflectance in *moderately* narrow spectral regions affects color discrimination. Man distinguishes up to 150 spectral hues. For bees, however, as has been said, there are only four; within the range of each of these four no discrimination appears to be made. For the same reasons, individual absorption lines, which are of vital importance in spectroscopy proper, may be disregarded; in behavior it is the general picture that matters.

3. The apparatus used in the field laboratory must be sturdy and not subject to special operational difficulties in a humid tropical climate.

4. Since the wings of many of the species to be studied had complex wing patterns and colors, and time for the problem was limited, the method chosen must be fairly rapid.

Three general approaches were possible:

1. *Spectroscopic.* A quartz instrument is essential for this method, because of the necessity of determining reflectance in the near ultraviolet, which is not sufficiently transmitted by ordinary glass. Suitable small instruments, such as described by Richtmyer (1923, p. 153) are apparently not being manufactured today. The search was early given up, however, because it was realized that, in the study of butterfly wings, many of the areas of greatest interest were too small to be readily analyzed by their reflected light, especially with the small instrument that would be taken to the field laboratory. Also, the simple photographic laboratory facilities available were not suitable for the delicate processing of spectographic plates.

2. *Spectrophotometric.* There were four disadvantages to this method, which, under ideal conditions, would be the best. First, even the largest and most modern instruments are not suitable for the analyses of the reflectance of very small areas; samples about 4 centimeters

square are desirable, while those less than one centimeter square can scarcely be handled. The majority of our specimens, even flowers, do not meet these requirements. Second, it is difficult to control the scattering of light, especially in the short-wave end of the spectrum, in such samples of diffuse reflectance as insect wings. In these at least some of the color is often structural, instead of completely pigmentary. Also, because of the overlapping scales, the surface is not altogether smooth. Third, in humid tropical weather, spectrophotometers may need highly technical servicing which is not available; among non-physicist operators, the development of instrument untrustworthiness might not be promptly or easily detected, much less corrected. Fourth, except in the largest recording instruments, which are beyond the means of small research laboratories requiring only their occasional use, the analysis of each colored area may require many hours. Therefore, it was finally decided that the method was both too precise and yet too subject to error for field use by amateur spectrophotometrists for the rather coarse determinations desired. Zoological workers interested in the possible applications of spectrophotometry to their own problems will find in Harrison, Lord & Loofbourow (1948) a comprehensive introduction to the subject.

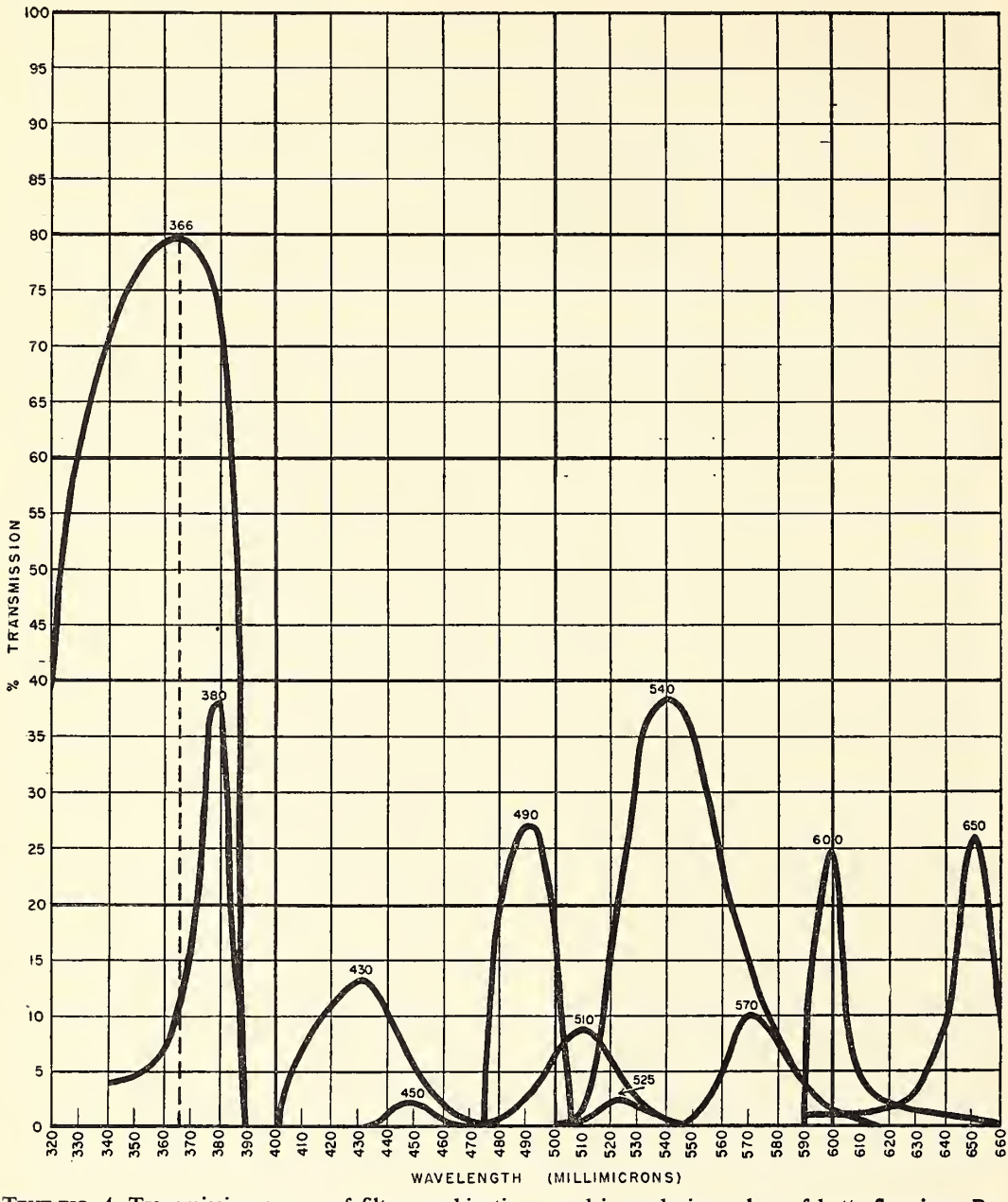
3. *Photographic.* For the reasons stated above, the relatively simple method of photographic filter analysis was selected. This is essentially an elaboration and refinement of that used by Lutz in his preliminary analysis of ultraviolet reflectance in flowers (1924).

The exact method eventually perfected in the present investigation is, in outline, as follows: Wings or parts of wings were photographed through a series of narrow-band-pass and interference filters and filter combinations (Table 1, Text-fig. 4). For preliminary surveys of several entire wings, crowded on single negatives at high reductions, a small area of magnesium oxide was included as a standard (Pl. II). This compound reflects a maximum of light falling on it of all wavelengths including the near ultraviolet. The densities of the photographed samples in each negative could then be roughly compared by visual inspection with the density of the magnesium oxide standard in the same frame. Small areas of color which appeared of special interest could subsequently be cut out of the wing, and rephotographed at higher magnifications.

For this second photographing, small samples were included in each negative of 19 Munsell neutral gray papers, ranging in value from "black" to "white." Upon our return to New York from Trinidad, the resultant negatives were analyzed by means of a microdensitometer, the reflectance of the specimens in each frame being matched in density with a gray sample of similar reflectance in the same frame (Pl. III). Spectrophotometric curves were secured for the gray scale (Text-fig. 5). Thus the reflectance of a given specimen at the points of peak transmission of the various filter combinations could then be read directly from these spectrophotometric curves. In this way a percentage was determined of the reflectance of the specimen in each spectral region. Obviously, the method is rough, when judged by the standards of modern spectroscopy. However, when applied with maximum care and with sufficient checks, the procedure has proved wholly adequate for our purposes. It has the advantage that prompt macroscopic inspection of the results may be made in the field, as a preliminary to experiments with hitherto untested species, without waiting for densitometric analysis. It is clear at once, for example, on any negative strip, whether or not any noteworthy amount of ultraviolet reflectance is present, and whether a certain red extends strongly down into the yellow-orange or yellow. If it does so extend, for those insects which are known to have weak sensitivity in the red region, the patch could still be strongly perceived as a hue. A second reason for analyzing the wings in the field rather than from mounted museum specimens is that the possible effects of drying and of chemical insecticides have been heretofore unkown.

The method will now be described in detail, along with the chief sources of error and the necessary precautions that must be observed.

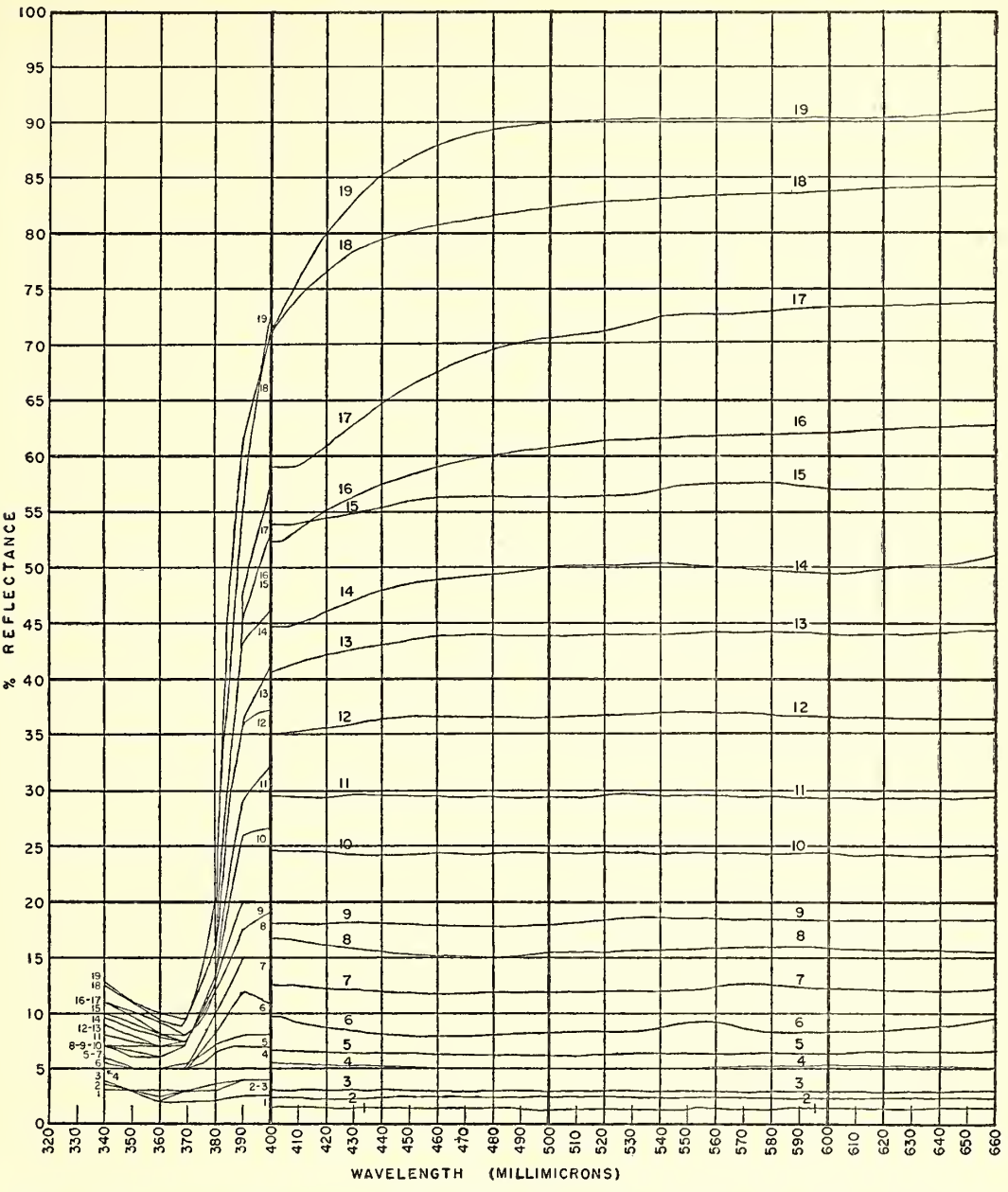
A. *Selection and Preparation of Material.* Except where the effects of aging and fading were being studied, fresh, unmarked specimens were used. Where possible, those less than four days out of the pupa were selected, which was often feasible, thanks to the Simla laboratory's insectaries (Crane & Fleming, 1953). The butterflies were killed by pinching the thorax, in order to eliminate possible effects of chemicals on spectral reflectance, and were photographed at once. When photography by sunlight was necessary, the desired areas of wing were cut out and, taking care to handle them only by the extreme edges with fine forceps, were attached to black matte paper with rubber cement; this was necessary because of the wind. Indoors, the specimens were simply arranged on black velvet, on a stage or table immediately beneath and at precisely right angles to the lens. All species discussed in this paper have been analyzed at least twice and frequently more often, at various magnifications, so that the results presented are well checked.



TEXT-FIG. 4. Transmission curves of filter combinations used in analyzing colors of butterfly wings. Redrawn from spectrophotometric curves furnished by the Electrical Testing Laboratories, and Bausch and Lomb Optical Co. See Table 1 for identification of filters. The vertical broken line at 366 mμ indicates position of the peak of output, a narrow band, of the ultraviolet lamp employed, "Mineralight" SL 3660, after a curve furnished by the manufacturer (Ultraviolet Products Co.).

B. *Magnesium Oxide Standard.* In the preliminary survey of entire wings under low power, a sample of white magnesium oxide was included along the edge of each negative. Since this compound reflects about 98 percent of visible light at any given wavelength, in both the visible and near ultraviolet, it is a convenient gauge of relative brightness of the objects photographed. When an attempt is made to photograph the standard in each negative of a series to a similar and fairly high density, the relative density of a given piece of wing photographed through each filter combination can be roughly estimated by inspection.

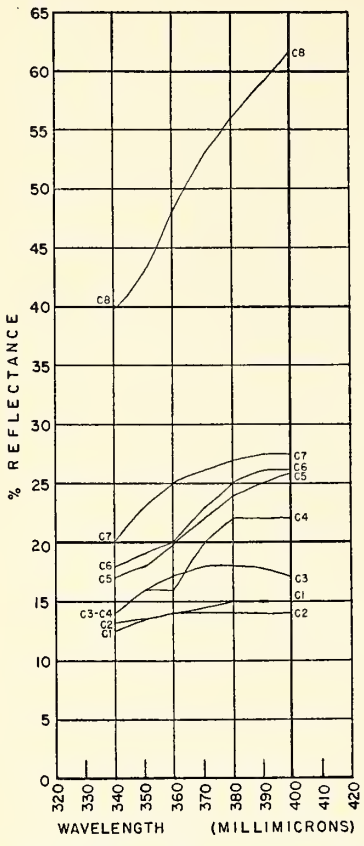
An example is given in Plate II, Fig. 42. The six frames, reading from left to right, were ex-



TEXT-FIG. 5. Reflectance characteristics of neutral gray papers used for comparison standards in butterfly color analyses. Redrawn from spectrophotometric curves furnished, in the visible, by the manufacturer, Munsell Color Co., and in the ultraviolet by the research laboratories of the Interchemical Corporation. The numbers from 1 to 19 indicate the individual papers from darkest to lightest, and correspond, in that order to the following Munsell identification numbers: N1, N1.5, N2, N2.5, N3, N3.5, N4, N4.5, N5, N5.5, N6, N6.5, N7, N7.5, N8, N8.5, N9, N9.2, N9.6.

posed through filters or filter combinations with peaks in, respectively, the ultraviolet, blue-violet, blue-green, green-yellow, yellow and orange-red. The left image in each negative frame represents the under wings of *Callicore aurelia,* in which most of the under forewing is red. Even in this printed reproduction, it is at once evident that the area has low reflectance in the ultraviolet and violet-blue, practically zero in the blue-green and yellow-green, low in the yellow and moderately high in the orange-red.

Accurate percentages, however, of reflectance in terms of magnesium oxide cannot be obtained by direct comparison of the densities of the spec-



TEXT-FIG. 6. Ultraviolet characteristics of certain Stoelting Psychological Test Papers for use in supplementing gray standards. Redrawn from spectrophotometric curves furnished by the research laboratories of the Interchemical Corporation. Correspondence of identification numbers: C1 = Stoelting No. 6; C2 = Stoelting No. 7; C3 = Stoelting No. 16; C4 = Stoelting No. 15; C5 = Stoelting No. 12; C6 = Stoelting No. 14; C7 = Stoelting No. 11; C8 = Stoelting No. 17.

imen with that of the standard through use of the densitometer. This is because of fundamental characteristics of the photochemical process, in which faint light affects the density of a negative relatively less than does strong light. As a result, length of exposure and time of development also have unequal effects on latent images of highly different potential densities; the necessary control of conditions, to insure uniformly of negative material and its processing and proper analysis of the results, was beyond the scope of the facilities at hand. Instead, a gray series, as described below, was included in each negative wherever exact numerical ratios were desired.

For use in the preliminary analyses, the magnesium oxide standard was prepared. The stainless steel blade of a table knife was coated with magnesium oxide in the following manner: A small heap of magnesium metal was ignited with matches. The knife was then easily coated in the smoke, which could be stimulated by poking the embers with a dissecting needle. The method, although simplicity itself, gave trouble to our group of non-chemists until we discovered that a heap of the metal, rather than a flat patch, is needed for easy ignition. The match should be thrust into a hollow at the top of the heap. The knife must be recoated at least every three days, if in daily use; if more seldom, immediately before every photographic session. The special standards which can be purchased from the U. S. Bureau of Standards for spectrophotometric work were not necessary for the rough results desired.

C. *Gray Standards.* In each negative intended for accurate analysis were placed samples of each of the 19 steps in the Munsell Neutral Gray series. They were attached to strips as follows: Small rectangles were cut from the gray papers, of such a size that in the finished negative each measured at least 2 mm across. Smaller sizes were difficult to measure on the densitometer, although samples as small as 1 mm square could be used where necessary. These rectangles were divided into two groups and attached with paper cement to two strips of cardboard; a number designating each step was written underneath in India ink, to be visible on the developed negative. Care was taken to handle the grays only by the edges and with forceps; the strips were kept in light-tight boxes in cellophane envelopes when not in use. In composing a frameful of sample wing areas under the lens, a strip was placed along one or both long edges of the field, the specimens being laid between them. This arrangement made for ease of comparison both by inspection and in the densitometer (Pl. III, Figs. 45-52).

Spectrophotometric analyses in the visible were obtained from the manufacturers of the actual gray papers used (the Munsell Color Co., Baltimore, Maryland). The ultraviolet reflectance of the papers was furnished, from 340 to 400 mμ, by the Research Laboratories of the Interchemical Corporation (Text-fig. 5). Since reflectance in this region proved to be exceedingly low, with sharp drops below the violet, they were supplemented for ultraviolet exposures by a series of seven colored rectangles, selected for their progressively higher ultraviolet reflectance curves, from the Stoelting Psychological Color Test series (Pl. III, Figs. 45, 46). Their ultraviolet reflectance curves were also obtained from the Interchemical Corporation (Text-fig. 6).

D. *Camera and Lenses.* The most satisfactory set-up proved to be a Leica camera equipped

with a reflex housing and the front element of the 135 mm Elmar lens fastened to the Leitz bellows attachment. This assembly, in conjunction with a "Highboy" tripod, gave maximum ease of focussing and adjustment, under the circumstances, since the set-up could not be left indefinitely in place because of other uses for the equipment. A maximum magnification of 1:1.1 was obtained without the addition of extension tubes. The minimum area which could be accurately analyzed in the standardly equipped densitometer was about 1 mm square. The density of the smaller lines and spots could be roughly gauged by inspection. The specimens and comparison material were placed on a piece of black velvet beneath and at right angles to the vertically-aligned camera. A 90 mm Elmar lens in combination with extension tubes and a ground-glass focussing assembly were also used with success, although this set-up proved much less convenient. Both the 90 mm Elmar and the 135 mm Elmar adequately transmitted ultraviolet in the desired region.

E. *Filters.* During the first season of 1950, a preliminary series of six combinations of Eastman Wratten filters was used. With appropriate substitutions and additions, this was expanded between 1951 and 1953 into a series of twelve combinations, including three Bausch and Lomb interference filters. The latter, used in conjunction with Eastman filters, transmitted with a narrow peak at 380, 600 and 650 mμ, respectively. They proved to be a satisfactory answer to the problem of isolating narrow bands in these regions, which could not be adequately dealt with by combinations of the regular glass and gelatin filters. The transmission curves of the combinations of the actual filters used were secured from the Electrical Testing Laboratories (Table 1, Text-fig. 4). The filters were attached to the camera by double or triple adapters when in use; at other times they were kept tightly closed in boxes. The basic tests were made within five months of the time the filters were spectrophotometrically analyzed. The selected filters are designated by the manufacturer as moderately stable and very stable. Their curves compare extremely closely with those furnished by Eastman in "Wratten Light Filters" (Anon., 1945).

F. *Film.* Throughout the series, Eastman 35 mm Plus X film was used. In this film, the spectral sensitivity extends far into the ultraviolet but, at the exposures used, not beyond 650 mμ in the red. Since no frame was being compared directly with any other frame, but only with a gray sample of similar density within the same frame, no special precautions were necessary to insure uniformity of the film used, temperature at which it was stored, etc.

G. *Illumination.* (Pl. III, Fig. 53). For the visible part of the spectrum, a single General Electric 2A photoflood lamp, with built-in reflector, was used. Because of the small size of the field, one lamp proved sufficient for even illumination. It was placed at an angle of 45 degrees to the camera-specimen axis, at a distance of 2½ to 3 feet from the specimen. Extreme care was taken to maintain this angle, in order to avoid direct reflectance of the light from any smooth scale surfaces into the camera, which of course occurs when a lamp is placed close to the camera-subject axis—that is, close to a 0° angle of incidence. In the early tests, before adequate precautions were taken, high reflectances were sometimes recorded in regions which, when properly tested subsequently, showed almost negative results.

The 45° angle of incidence was also maintained for areas in which the color was due at least partly to the structure of the scales. In the red of *Callicore, Biblis* and *Papilio,* for example, the iridescent effect was scarcely visible at this angle. (see p. 108). In strongly iridescent areas (*Morpho, Caligo*), the selected angle resulted in a preponderance of moderately short waves of moderate intensity; if the angle had been decreased, the result would have been a higher intensity of color with a preponderance of somewhat longer wavelengths, in accordance with the laws governing interference colors (e.g., Richards, 1951, p. 197 ff.; Fox, 1953, p. 56 ff.; and present paper, p. 109).

Lamps were replaced frequently, that is, before their weakening necessitated changed exposures. A black cloth hung between camera and lamp in the otherwise darkened room, and an effective sunshade, minimized the problem of scattered light. Incidentally, the cloth, even though black, protected the camera from the heat of the nearby lamp.

For the ultraviolet region, exposures under both a lamp and sunlight were made. The lamp was a model SL3660 "Mineralight" manufactured by Ultra-Violet Products Inc., South Pasadena, California. It had a narrow peak output at 366 mμ. Its use in conjunction with the Wratten 18A filter assured a limitation of the light to the desired region. The lamp was mounted on a horizontal standard which could be swung into position, replacing the Photoflood, for the 366 mμ ultraviolet exposure in each series; the same precautions to ensure a 45° angle are of course as essential as in the case of the visible light.

All specimens were also checked in bright sunlight, in combination first with the 18A filter,

giving a rather broad ultraviolet transmittance band, and second with this filter combined with an interference filter transmitting at 380 mμ. This combination of indoor and outdoor work insured against failure to discover strong areas of ultraviolet reflectance between 366 mμ and the visible. The outdoor work was restricted to those checks and to some preliminary surveys, because of the great difficulties due to breezes, changing light and stray reflections. In the final ultraviolet checks outdoors, the photography was confined to bright sunshine in mid-morning or mid-afternoon, in the middle of a wide-open gray-slate terrace. Even under these optimum conditions, however, scattered ultraviolet light was such a large factor out-of-doors, that strongly iridescent areas could yield no reliable comparative data and so are omitted from Table 3. These sunlight records of iridescent and partly iridescent surfaces were, however, useful in giving some ultraviolet maxima recorded under natural lighting conditions, and are included in the systematic section under the species concerned. Even areas of partly structural white, such as the wings of *Eurema albula,* were impossible to light evenly out-of-doors, as is shown in Pl. III, Fig. 45.

H. *Exposures.* The exposures by artificial light with the various filter combinations varied from 1/20 sec. to 30 sec. at stops of f 5.6 to f 8. The aim was simply to obtain an image of the desired specimen, at each filter combination, dense enough for easy comparison in the densitometer with the standard gray samples appearing in the same negative. This method obviated the necessity for attempting to equalize densities in respect to a magnesium oxide standard. As has already been mentioned (p. 93), a simple comparison of the density of an image of the specimen to that of a magnesium oxide standard in each negative could not be made to yield accurate results. Hence the necessity of using the gray series. Satisfactory sunlight exposures were around ⅛ sec. at f 9 for the 18A filter, ¼ sec. at f 9 for the 380 mμ interference filter in conjunction with the 18A filter, all being made with the bellows almost fully extended.

I. *Development.* All negative strips were developed in a roll-type tank in Eastman Microdol at standard times and temperatures with the usual precautions to obtain fine grain and even development.

J. *Densitometer.* The Welsh Densichron was used for eventual analysis of the negatives, the minimum aperture being selected for passing the beam of light up through the stage. This permitted, as described earlier, density readings from areas measuring a minimum of 1 mm square. It was found helpful to slip a piece of white paper temporarily between the negative and the aperture, for ease in the precise centering of tiny crucial areas above the slender beam of light. Several readings were taken for each area and, in the event of slight differences, the range recorded; the results were matched as closely as possible with a sample gray in the same negative, its reading being also noted as either more or less than that of the specimen. Because of the uneven nature of butterfly wings and their scales, it was sometimes necessary to use cautious judgment in selecting as typical a particular tiny area of a fairly large negative image for densitometric analysis; this was especially true of whole wings, which could seldom be completely flattened, and was an especial problem, because of the increased difficulty with scattered light, in ultraviolet photography out-of-doors (see under *Illumination* and Plate III, Fig. 45).

The final step was the determination of the percentage reflectance of the sample in each spectral region in terms of magnesium oxide. This was accomplished merely by reading, from the spectrophotometric curves of the Munsell gray samples, the percentage reflectance in each matching sample at the point of peak transmission by the filter combination used for a particular negative. In the case of high ultraviolet reflectance, the spectrophotometric curves of the selected Stoelting colors (p. 94) were consulted instead of the Munsell gray series.

Systematic Section

Note: The terms "negative reflectance," "negatively ultraviolet," etc., are used to denote reflectance values lower than the reflectance in the corresponding spectral region of the darkest standard gray paper—that is, less than 1.5% of the reflectance of magnesium oxide (see Text-fig. 5).

Black areas show negative reflectance in the ultraviolet, just as in the visible; they will not be mentioned in this section.

"Positively ultraviolet white" indicates high reflectance in the near ultraviolet, approaching that of magnesium oxide in this region, and approximately equal to the specimen's reflectance in the visible.

FAMILY DANAIDAE

Danaus plexippus megalippe (Huebner)
(Plate I, Figure 1)

Lycorea ceres ceres (Cramer)
(Plate I, Figure 2)

The spectral characteristics of these two species are practically identical and may be listed as follows:

1. The small white spots on the margins of

the wings and on the thorax are very strongly positively ultraviolet, with comparable reflectance throughout the spectrum. Therefore, these whites are "pure" for these ultraviolet-sensitive insects.

2. In both the browns and the yellows, the ultraviolet reflectance is negligible on the upper wing surfaces, ranging from negative to less than 4%, the higher values occurring in ♀♀. In both sexes the reflectance is higher on the underwings than on the upper, corresponding to their paler coloration in the visible region.

3. Violets and blues are negative in both the browns and yellows.

4. The yellows take their first strong upward curve in the low blue-green, about 490 mμ; the browns on the other hand do not reflect markedly until around 525 in the green.

5. In the longer wavelengths, from 590 to 650, both yellows and browns reflect strongly, although the yellow is stronger than the brown except up in the orange-red where they finally approach equality.

6. For practical purposes, therefore, these yellows differ from the browns, first, in including a moderately strong blue-green component and, second, in their generally higher reflectance in the green, yellow and orange. The colored abdominal streaks do not differ noticeably in spectral composition from those of the wings.

FAMILY ITHOMIIDAE

Tithorea mopsa megara (Godart)
(Plate I, Figure 3; Plate II, Figure 43, right image in each frame)

Mechanitis doryssus veritabilis Butler
(Plate I, Figure 4)

Hypothyris euclea euclea (Godart)
(Plate I, Figure 5)

Hypoleria ocalea
(Doubleday, Hewitson & Westwood)
(Plate I, Figure 6)

Ithomia drymo pellucida Weymer
(Plate I, Figure 7)

Hymenitis andromica trifenestra (Fox)
(Plate I, Figure 8)

These six species are divided by color into two groups. The first four include all those combining brown, yellowish and black in their patterns, and usually having small white marginal spots. In spectral characteristics they are practically identical with the danaids described above.

The second group, composed of the largely transparent *Ithomia* and *Hymenitis*, show no interesting color characteristics. The transparent areas do not reflect ultraviolet (except, of course, when the membrane directly reflects incident light); the terminal white forewing spots and body spots of *Hymenitis* and the single forewing spot of *Ithomia* are positively ultraviolet. The brown marginal markings of both species are negatively ultraviolet.

FAMILY SATYRIDAE

Euptychia hermes (Fabricius)
(Plate I, Figure 9)

Euptychia hesione (Sulzer)
(Plate I, Figure 10)

The above two species of the genus *Euptychia* are typical in general appearance of the numerous local species of inconspicuous brownish wood nymphs. These, in addition to four other species examined more cursorily, show similar spectral reflectance characteristics. Because of their lack of spectral interest and variety the group was not analyzed in detail. All of their whites and very pale colors are positively ultraviolet, including the perimeters and highlights of the ocelli. The browns are typical of that color, showing practically negative reflectance in the ultraviolet, violet, blue and blue-green, and moderately weak reflectance in the longer wavelengths.

FAMILY HELICONIIDAE

Heliconius numata ethilla Godart
(Plate I, Figure 11; Plate II, Figure 43, left image in each frame)

Browns, yellows and white spots indistinguishable from those of the danaids (p. 96) and similarly colored ithomiids (above) previously discussed.

Heliconius melpomene euryades Riffarth
(Table 3)

Heliconius erato hydara Hewitson
(Plate I, Figure 12; Plate III, Figure 46; Text-figure 9a; Tables 2, 3)

The characteristics of the red forewing band of the very common *H. erato hydara* and of the locally exceedingly rare *H. melpomene euryades* appear identical, both to the human eye and in spectral reflectance, as does the corresponding pale pinkish band of the underside. Therefore the two species are here considered together. The reddest and brightest bands were Scarlet Red (Ridgway).

Reflectance in the ultraviolet is uniformly low, regardless of sex, freshness or narrow spectral band. The gross variation is from negative reflectance to 5%. The average reflectance from 10 specimens of *H. erato* (6 ♂♂, 4 ♀♀) is about 2.5% or less. The higher reflectances are sometimes found in the 366 mμ band, sometimes in

the 380 mμ band; 5% was attained twice: once by a moderately worn ♀, analyzed only after two years, at the 380 mμ band, and once by a ♂, freshly emerged and promptly photographed, at the 366 mμ band. The minima were found in similar extremes of the material—worn but promptly photographed ♀♀, fresh ♂♂ photographed after two years, etc.

The reflectance continues very low (less than 2%) through the violet, blue, blue-green and green, the blue-green (490-510 mμ) invariably registering minimum readings.

In the yellow-green (around 540 mμ) the reflectance mounts to about 3%, and from there on there is a steady rise to about 30% in the orange-red (650 mμ).

No sexual differences have emerged. The noticeably oranger red (often Peach Red of Ridgway) of older specimens is recorded in the spectral analyses by slightly higher readings in the yellow and orange. However, the rise does not begin at shorter wavelengths, or, in other words, faded specimens do not reflect blue-green any more than do fresh ones.

The underwing band, more or less pinkish white to the human eye, often unevenly so, strongly reflects all wavelengths including the ultraviolet, with a very slight rise at the long end of the spectrum. (*Note*: The underwing bands in Surinam specimens of *H. erato* and *H. melpomene* are much redder than in the Trinidad forms and have low reflectance in the ultraviolet).

The red dots near the bases of the underwings are indistinguishable from the upper forewing band. The yellow streaks on the head, thorax and along the basal fore margin of the under hindwing are negatively ultraviolet, and resemble in general reflectance pattern the upper forewing band of *H. sara* (see below).

Heliconius sara rhea Cramer

(Plate I, Figure 13; Plate III, Figures 47-50, 2nd image from left in each frame; Text-figure 8a; Tables 2, 3)

Yellow spots on upper forewing: (Usually Martius Yellow of Ridgway). Ultraviolet almost negative, violet low, blue moderate, a sharp climb in the low blue-green which then holds almost level at around 50% through the orange-red, there being a slight maximum in the yellow-green and yellow.

Iridescent blue on hindwing: (Blue Violet to Hyacinth Blue of Ridgway). Moderate and about equal reflectance in the ultraviolet, violet and blue; reflectance reduced but present in blue-green, practically negative at longer wavelengths.

Yellowish white on lower forewing and on body: Strong reflectance at all wavelengths, including ultraviolet.

Red dots on lower hindwing about as in *H. erato hydara.*

No conspicuous sexual differences.

Heliconius ricini insulana Stichel

(Plate I, Figure 14; Plate III, Figures 47-50, 3rd image from left in each frame; Text-figure 9b; Tables 2, 3)

Yellow spot of upper forewing: (Martius Yellow of Ridgway). Similar to that of *H. sara rhea* (see above).

Red area of upper hindwing: (Scarlet Red of Ridgway). Similar to the forewing red band of *H. erato hydara* (p. 97).

Pale yellowish markings of underwings and body: As in *H. sara rhea.*

Heliconius aliphera aliphera (Godart)

(Plate I, Figure 15; Plate II, Figure 44, left image in each frame)

Orange of upper wings: (Zinc Orange of Ridgway; dull specimens Tawny). Reflectance practically negative in the shorter wavelengths, including the ultraviolet, through the blue-green; a steep rise starts in the green and continues through the yellow, orange and orange-red.

Paler undersides of wings similar except for the usual moderate reflectance throughout the shorter wavelengths including the ultraviolet.

Dryas julia julia (Fabricius)

(Plate I, Figure 16; Text-figure 8b; Tables 2, 3)

Orange of upper wings: (Orange Rufous to Ochraceous Orange of Ridgway). Practically identical with *H. a. aliphera* (see above), except that the richest areas in fresh specimens tend to have higher values in the orange and red.

Undersides of wings about as in *H. a. aliphera.*

Agraulis vanillae vanillae (Linnaeus)

(Plate I, Figure 17; Plate II, Figure 44, middle image)

Orange of upper wings: (Orange Rufous to Zinc Orange of Ridgway). Practically identical with *H. a. aliphera* and *D. j. julia* (see above) except that there is somewhat higher reflectance in the yellow-green and yellow.

Undersides of wings: The silvery-white spots reflect maximally in all regions including the ultraviolet. Remainder of surface about as in *H. aliphera,* except that there is almost negative reflectance in the ultraviolet and violet.

FAMILY NYMPHALIDAE

Phyciodes ofella ofella (Hewitson)
(Plate I, Figure 18)

Phyciodes leucodesma (Felder)
(Plate I, Figure 19)

The whites in both species are positively ultraviolet; the brownish blacks are unremarkable.

Victorina steneles steneles (Linnaeus)
(Plate I, Figure 21; Text-figure 7c; Table 2)

Green of upper wings: (Light Yellow Green to Lumière Green of Ridgway, fading to Oural Green and Lichen Green). Reflectance in all specimens moderately low (8%) in ultraviolet, very low in violet and blue, and more than equal to ultraviolet in the blue-green. Nearly equal maxima extend from the green through the orange, with a slight decline in the orange-red. Faded specimens show slightly higher values in the visible short-wave regions.

Pale green of under wings similar to that of upper, but with higher reflectances throughout.

Ochraceous Orange (Ridgway) markings of under wings with negative reflectance at wavelengths shorter than green.

White markings positively ultraviolet.

Biblis hyperia (Cramer)
(Plate I, Figure 22; Text-figure 9c; Table 2)

Red of upper hind wing: (Scarlet Red of Ridgway, fading to Scarlet; a faintly iridescent blue film). Reflectance in ultraviolet higher than in any other region except yellow through orange-red; violet and blue moderately low; blue-green negative; green low; yellow-green moderately low; a sharp climb starting only at orange, continuing through orange-red. At larger angles of incidence there is a much higher—up to 20%—reflectance in the ultraviolet, which is indicated in the visible only by the transparent bluish structural sheen overlying the red pigment. The iridescent sheen is more apparent in some individuals than in others.

Colobura dirce dirce (Linnaeus)
(Plate I, Figure 24)

Dynamine theseus (Felder)
(Plate I, Figure 25)

Dynamine artemisia (Fabricius)
(Plate I, Figure 26)

The whites are positively ultraviolet and the browns unremarkable in all three species.

Adelpha cytherea insularis Seitz
(Plate I, Figure 28; Plate II, Figure 42, image at right in each frame)

Adelpha iphicla daceleia Fruhstorfer
(Plate I, Figure 27; Plate II, Figure 52, middle image in each frame)

In both species the slightly opalescent whites of the upper wing surfaces are positively ultraviolet. Orange (Mars Yellow of Ridgway) markings negative in ultraviolet and violet; a rise in reflectance starts in the upper blue-green; reflectance strong from yellow-green through orange-red.

Browns and undersides unremarkable.

Protogonius hippona trinitatis Rober
(Plate I, Figure 29)

Browns spectrally closely similar to those of the danaids, ithomiids and heliconiids.

Callicore aurelia (Guenée)
(Plate I, Figure 23; Plate II, Figure 42, left image in each frame; Text-figure 9d; Table 2)

Predominantly greenish iridescence of upper forewing: (Visually ranging from pale blue to green yellow). A typical spectral pattern, with angle of incidence at 45°, shows moderate reflectance in the ultraviolet and violet, while the level is twice as high and about equal from the blue throughout the orange-red, except for a dip in the blue-green.

Red of under forewing: (Spectrum Red to Rose Red of Ridgway, with overlying bluish structural film). Ultraviolet reflectance low to moderately low, the higher values at larger angles of incidence, violet low, blue moderately low, blue-green and green negative, green-yellow and yellow low; a very sharp climb starts in the orange and continues upward through the orange-red.

White of under hindwing: Positively ultraviolet.

Anartia amalthea amalthea (Linnaeus)
(Plate I, Figure 20; Text-figure 7d; Tables 2, 3)

Orange-red of upper wings: (Brazil Red of Ridgway, fading through Orange Rufous to Apricot Buff). Spectral reflectance negative in ultraviolet, regardless of whether fresh or faded; violet and blue very low; blue-green and green negative, green-yellow and yellow very low; a steep rise starts abruptly in the orange, and levels off in the orange-red. Faded specimens show higher values in the greens and yellows.

Browns of undersurfaces unremarkable.

FAMILY MORPHIDAE

Morpho peleides insularis Fruhstorfer
(Plate I, Figure 30; Text-figure 7a; Table 2)

TABLE 2. REPRESENTATIVE SPECTRAL REFLECTANCE PATTERNS OF SELECTED BUTTERFLY WING AREAS
(See also Text-figs. 7-9 incl.)

Subject [1]	Location	Color	% Reflectance (mμ) [2]									
Peak transmission of filter combinations (see Table 1 & Text-fig. 4)			366 [3]	430	450	490	510	525	540	570	600	650
HELICONIIDAE												
Heliconius erato ♂	upper forewing	red	3.5	2	2	neg.	neg.	2	3	9	19	29
Heliconius erato ♀ (faded)	upper forewing	red	1.5	2	2	neg.	neg.	4.5	5	14	19	29
Heliconius sara ♂	upper forewing	yellow	3	9	25	37	48	49	52	50	48	48
Heliconius sara ♀	upper forewing	yellow	3	8	20	44	48	48	50.5	47	45	44
Heliconius ricini ♂ (faded)	upper forewing	yellow	3	11	19	17	44	40	30	35	38	31
Heliconius ricini ♂	upper hindwing	red	2	neg.	2	neg.	neg.	1.5	6.5	12	22	31
Dryas julia ♂	upper forewing	orange	neg.	neg.	neg.	neg.	neg.	7	19	24	27	38
NYMPHALIDAE												
Victorina steneles ♂	upper forewing	green	8	3	4	9	15	18	20	20	20	18
Biblis hyperia ♂	upper hindwing	red [4]	9	3	5	neg.	neg.	2	6.5	9	29.5	36
Callicore aurelia ♀	under forewing	red [4]	6	3	5	neg.	neg.	neg.	2	3	14	40
Callicore aurelia ♀	upper forewing	green [5]	12	13	25	17	25	26	26	26	25	23
Anartia amalthea ♂	upper hindwing	red	neg.	3	2	neg.	neg.	neg.	5	3	21	25
MORPHIDAE												
Morpho peleides ♂	upper forewing	blue [5]	16	30	38	21	20	20	12	8	8	2
BRASSOLIDAE												
Caligo illioneus ♂	upper hindwing	violet [5]	11	5	13	6	5	5	5	4.5	3.5	2.5
PAPILIONIDAE												
Papilio neophilus ♂ (faded)	upper forewing	green	neg.	9	15	12	17	16	17	14	19	14
Papilio neophilus ♂	upper hindwing	red [4]	9.5	2	neg.	2	1.5	neg.	3.5	9	24	33
Papilio neophilus ♀	upper hindwing	red [4]	14	10	8.5	neg.	neg.	neg.	1.5	5	18	24
Papilio anchises ♂ (30 yrs. old)	upper hindwing	red [4]	3	7	4	neg.	neg.	2	4	6	24.5	40
Papilio anchises ♀	upper hindwing	red [4]	5	4	3	neg.	neg.	2	3.5	10	25	47
Papilio anchises ♀ (1 yr. old)	upper hindwing	red [4]	3	3	5	neg.	neg.	2	3	9	20	35
Papilio anchisiades ♂	upper hindwing	red [4]	9	7	5	2	2	2	4	8	18	24.5
Papilio anchisiades ♀	upper hindwing	red [4]	8	5	5	2.5	2	4	5	9	15	24.3
Papilio thoas ♂	upper hindwing	yellow	neg.	2	6	11.5	15	19	21	27	29	29
PIERIDAE												
Anteos maerula ♂	upper hindwing	yellow	neg.	neg.	2	24	38	42	42	44	46	49
Anteos maerula ♂	under hindwing	yellow	5	10.5	12	18	25	27	28	28	26	18
Phoebis sennae ♂	upper hindwing	yellow	2	3	9	36.5	36.5	49	50.5	50.5	61.5	44
Phoebis sennae ♂	under hindwing	yellow	6	11	14	36.5	36.5	50	52	52	56	42
Phoebis sennae ♀	upper hindwing	yellowish	17.5	25	27	25	25	29	29.5	33	30	28
Phoebis sennae ♀	under hindwing	yellowish	7	22	22	24	21	26	29	28	29	26
Melete lycimnia ♂	under forewing	white	5	38	44	52	55	53	50	58	62	70
Melete lycimnia ♂	under hindwing	yellow	neg.	8	15	46	47	47	48	57	60	68

[1] For subspecific names see Contents, p. 85, or systematic section. All specimens freshly killed and unfaded unless otherwise stated.

[2] "Neg." indicates less than 1.5%.

[3] Source = "Mineralight" SL 3660 lamp. See text, p. 95; cf. Table 3.

[4] Color due to red pigment overlaid with iridescent film. Spectral pattern given is typical for specimens lighted at usual angle, emphasizing pigment rather than iridescence; see text p. 95.

[5] Highly iridescent. Spectral pattern given is typical for specimens lighted at usual angle; see text p. 108.

TABLE 3. REFLECTANCES IN THE ULTRAVIOLET OF SELECTED BUTTERFLY WING AREAS
Photographed by Sunlight Only

		Locality	Age or condition	Time since killed	Exposed to Paradichlorobenzene	Area	Color	% reflectance with Wratten filter 18A (peak: 366 mμ)[1]	% reflectance with Wratten filter 18A + Bausch & Lomb interference filter (peak: 380 mμ)
HELICONIIDAE									
Heliconius melpomene	♂	Trinidad	unfaded	1 month	No	upper forewing	Red	Neg.	Neg.
Heliconius erato	♂	Trinidad	2 days emerged	just killed	No	upper forewing	Red	3.5	3
Heliconius erato	♂	Trinidad	unfaded	2 years	Yes	upper forewing	Red	3.5	3
Heliconius erato	♂	Trinidad	faded	1¼ year	Yes	upper forewing	Red	3.5	3
Heliconius erato	♂	Surinam	unfaded	1 month	Yes	upper forewing	Red	Neg.	Neg.
Heliconius erato	♀	Trinidad	2 days emerged	just killed	No	upper forewing	Red	2	2
Heliconius erato	♀	Trinidad	unfaded	2 years	Yes	upper forewing	Red	4	4
Heliconius erato	♀	Trinidad	faded	2 years	Yes	upper forewing	Red	1.5	2
Heliconius sara	♀	Trinidad	unfaded	just killed	No	upper forewing	Yellow	Neg.	Neg.
Heliconius sara	♀	Trinidad	unfaded	2 years	Yes	upper forewing	Yellow	Neg.	Neg.
Heliconius sara	♂	Trinidad	unfaded	just killed	No	upper forewing	Yellow	5	5
Heliconius ricini	♂	Trinidad	unfaded	2 years	Yes	upper hindwing	Red	Neg.	Neg.
Heliconius ricini	♂	Trinidad	unfaded	just killed	No	upper hindwing	Red	Neg.	Neg.
Dryas julia	♂	Trinidad	2 days emerged	just killed	No	upper forewing	Orange	Neg.	Neg.
NYMPHALIDAE									
Anartia amalthea	♂	Trinidad	unfaded	just killed	No	upper hindwing	Red	Neg.	Neg.
PAPILIONIDAE									
Papilio thoas	♂	Trinidad	unfaded	2 years	Yes	upper hindwing	Yellow	Neg.	Neg.
PIERIDAE									
Eurema albula	♂	Trinidad	unfaded	just killed	No	upper forewing	White	Neg.	Neg.
Eurema albula	♂	Trinidad	unfaded	just killed	No	under forewing	White	5	8
Eurema albula	♀	Trinidad	unfaded	just killed	No	upper forewing	White	9	9
Eurema albula	♀	Trinidad	unfaded	just killed	No	under forewing	White	15	16

[1] "Neg." indicates less than 1.5%.

Iridescent blue of upper wings: A typical spectral reflectance pattern, photographed with the usual angle of incidence of 45°, shows the highest values in the violet and blue; values are from about three-fifths to two-thirds as high in the ultraviolet and from the blue-green through the green; reflectance moderately low from the green-yellow through the orange, dropping almost to negative in the orange-red. Greater or lesser angles of incidence increase the intensities in the regions of shorter or longer wavelengths, respectively.

Undersurface, including ocelli, unremarkable, the whites being positively ultraviolet.

FAMILY BRASSOLIDAE

Caligo illioneus saltus Kaye

(Plate I, Figure 31; Text-figure 7b; Table 2)

Iridescence of upper wings: (Visual range from dull violet to dull blue). A typical spectral reflectance pattern, photographed as usual with an angle of incidence of 45°, is highest in blue, next highest in ultraviolet. Values one-third to one-half these maxima from blue-green through yellow, declining rapidly from there through the orange and orange-red. General changes with angles of incidence as in *Morpho*, but intensities much lower.

Undersurface unremarkable, the whites being positively ultraviolet.

FAMILY PAPILIONIDAE

Papilio neophilus parianus (Huebner)

(Plate I, Figures 33(♂), 34(♀); Plate II, Figure 44(♀), right image in each frame; Plate III, Figures 51, 52(♂), second image from right in each frame; Text-figures 9e(♂), 9f(♀); Table 2).

Green spot on ♂ forewing: (Light Blue-Green of Ridgway, fading to Deep Greenish Glaucous). Ultraviolet negative, violet low; a sharp rise in blue, followed by slight maxima in the greens and in the orange.

White spot on ♀ forewing: Positively ultraviolet.

Red spot of ♀ hindwing: (Tyrian Rose to Rose Red of Ridgway, fading to Old Rose, with a distinct transparent bluish iridescent film). Ultraviolet relatively high, higher than reflectance in any other region except orange and orange-red; violet and blue moderate, blue-green and green negative; yellow-green very low; yellow low, followed by a steep rise in orange and orange-red. At large angles of incidence the effect of the short waves of the structural film is intensified, which in the ultraviolet may surpass reflectance in the orange. The iridescent sheen is more apparent in some individuals than in others.

Red spot on ♂ hindwing: As in the ♀, but a purer red, except at large angles of incidence. Ultraviolet reflectance moderately lower than in ♀, violet, blue, blue-green and green all very low to negative; yellow-green low; yellow moderately high; a sharp climb in orange and orange-red, both of which are higher than in ♀.

Pink margins of upper hindwing and pinkish dots of under wings all positively ultraviolet and unremarkable. Red body markings about as in red forewing bands of *Heliconius erato* (i.e., structural film is lacking).

Papilio anchises cymochles Doubleday

(Plate I, Figure 32; Plate III, Figures 51, 52 third (♂) and fifth (♀) image from left in each frame; Text-figure 9g (♀); Table 2)

Green and white spots of forewing in ♂ and ♀ respectively as in *P. neophilus parianus*.

Red spots on hindwings, both sexes: (Scarlet Red to Scarlet of Ridgway). Very similar to ♂ of *P. neophilus parianus* except that the ultraviolet reflectance is low, ranging from negative to about 5% in both sexes, regardless of age. There is a slight iridescent film in this species also, but the increase in ultraviolet at large angles of incidence is less pronounced than in *P. neophilus parianus*.

White margins of hindwings positively ultraviolet.

Markings of under surfaces as in *P. neophilus parianus*.

Papilio anchisiades anchisiades Esper

(Plate I, Figure 36; Plate III, Figures 51, 52, first and second images from left in each frame; Text-figure 9h (♀); Table 2)

Very similar to *P. neophilus parianus* ♂ except that red spots of hindwings (Deep Rose Pink of Ridgway fading to Old Rose) tend to a very slightly lower ultraviolet reflectance, while the values from violet through the yellow-green are slightly higher, with no negatives.

Papilio thoas neacles Rothschild & Jordan

(Plate I, Figure 35; Plate III, Figures 47-50, image at left in each frame; Text-figure 8c; Tables 2, 3)

Yellow of upper wings: (Lemon Chrome of Ridgway, fading to Straw Yellow). Ultraviolet reflectance negative; violet and blue very low to low, followed by a moderately steep climb beginning in blue-green; a near maximum is reached in the yellow, and this level continues with only a slight subsequent rise through the orange and orange-red.

FAMILY PIERIDAE

Phoebis sennae marcellina (Cramer)

(Plate I, Figure 38; Text-figures 8e (♂), 8f (♀); Table 2)

Yellow of ♂ upper wing: (Greenish Yellow to Green Yellow of Ridgway). Ultraviolet very low, about 2%, violet and blue low to moderate; a strong climb beginning in the blue-green, continuing through orange, with a drop in the orange-red.

Creamy yellow of ♀ upper wing: (Pale Ochraceous Salmon of Ridgway to Warm Buff). Differs

Table 4. Areas of Butterfly Wings Showing More Than 5% Reflectance of Magnesium Oxide at 366 mμ, Angle of Incidence 45°
(White and pale-tinted areas omitted; percentages from Table 2)

Nymphalidae	
Victorina steneles: Upper wings; green	8 %
Biblis hyperia: Upper hindwing, marginal band; red pigment with iridescent film	9 %
Callicore aurelia: a. Under forewing, except apex; red pigment with iridescent film	6 %
b. Upper forewing, band; iridescent green	12 %
Morphidae	
Morpho peleides: Upper wings; iridescent blue	16 %
Brassolidae	
Caligo illioneus: Upper hindwing; iridescent blue-violet	11 %
Papilionidae	
Papilio neophilus: Upper hindwing, spot; red pigment with iridescent film ♂,	9.5%
♀,	14 %
Papilio anchisiades: Upper hindwing, spot; red pigment with iridescent film ♀,	8 %
Pieridae	
Phoebis sennae: a. Upper hindwing; structural and pigmentary creamy yellow ♀,	17.5%
b. Under hindwing; structural and pigmentary yellow ♂,	6 %
♀,	7 %

from ♂ upper wing in much higher values in the ultraviolet, violet and blue, but lower values from the blue-green through the orange-red.

Paler lower surfaces in both sexes with moderately low and similar ultraviolet reflectances, while the reflectances in other regions also tend more towards equality.

Anteos maerula maerula (Fabricius)

(Plate I, Figure 37; Text-figure 8d (♂); Table 2)

♂ only. Greenish yellow upper wings: (Green Yellow of Ridgway). Reflectance negative in ultraviolet and violet, very low in blue; a sudden steep rise occurs in blue-green, becomes gradual in yellow-green and reaches the maximum in the orange red.

Pale undersides of wings with higher values in the short wave regions, and lower values in the long, so that the regions approach equality and show only a slight maximum in the yellow-green and yellow.

Eurema albula (Cramer), form albula

(Plate I, Figure 39; Plate III, Figure 45; Table 3)

White of upper wings in ♂ with negative ultraviolet reflectance; ♀ with up to 9% in same region. Undersurfaces reflecting up to about 5% to 8% in ♂, around 15% in ♀.

Eurema venusta (Boisduval)

(Plate I, Figure 40; Plate III, Figures 47-50, large image at right in each frame)

♀ only. Pale yellow of upper wing surfaces reflecting about 8% ultraviolet, yellow under forewing about 7%, white under hindwing about 15%.

Melete lycimnia harti (Butler)

(Plate I, Figure 41; Table 2)

White under forewing of ♂: Ultraviolet 5%.

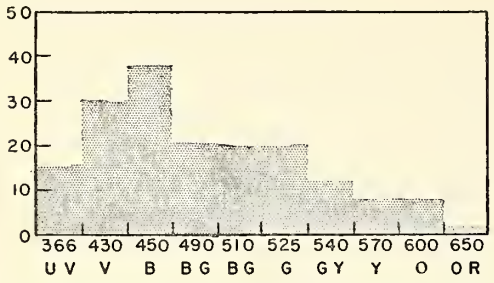
Yellow under hindwing of ♂: (Lemon Yellow of Ridgway). Ultraviolet negative, violet and blue moderately low to moderate, blue-green and green-yellow high and subequal; a steep climb in the yellow continues to rise through the orange-red.

Review of Spectral Regions

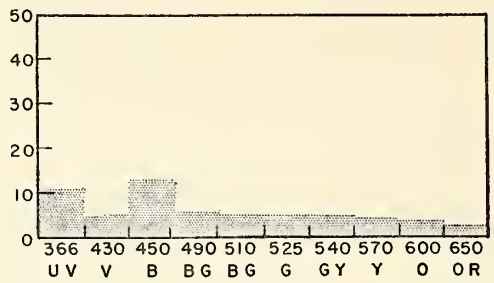
The material in the preceding systematic section will now be reviewed from the point of view of the principal spectral regions. Reference to Table 2, Text-figures 7, 8 and 9 and Plate I will be helpful in keeping the colors, spectral reflectances and appearance of the general wing patterns in mind.

A. Reflectance in the Ultraviolet Region

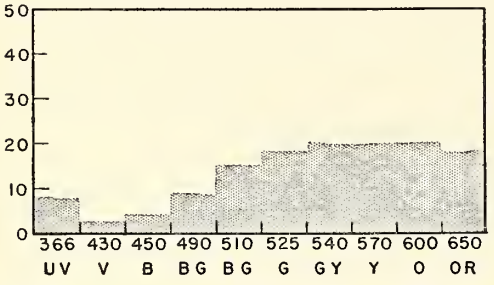
Forty-one species, belonging to 28 genera, have been surveyed in the preceding pages. Of these, only eight species show more than 5% of the reflectance of magnesium oxide at 366 mμ, in colors other than white or very pale tints. These species, all of which are included in Table 2, are listed in Table 4, along with the wing area showing the ultraviolet reflectance and its appropriate percentage at 366 mμ, when pho-



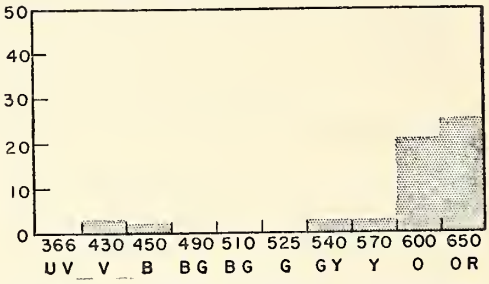
a. *Morpho peleides insularis* ♂: upper forewing, iridescent blue.



b. *Caligo illioneus saltus* ♂: upper hindwing, iridescent violet-blue.



c. *Victorina steneles steneles* ♂: upper forewing, green.



d. *Anartia amalthea amalthea* ♂: upper hindwing, red.

TEXT-FIG. 7. Reflectance characteristics of areas of special interest in selected butterflies. Typical, fresh examples illustrated. Cf. Tables 2 and 3, Text-figs. 8 and 9. Vertical coordinate, % reflectance in terms of magnesium oxide; horizontal, peak transmission of filter combinations employed, in millimicrons (see Text-fig. 4 and Table 1). Angle of incidence, 45°.

tographed with the incident light at an angle of 45°. Both sexes are similar in ultraviolet reflectance unless otherwise stated. The remaining species, reflecting less than 5% ultraviolet, have such strong reflectances in the visible that it does not seem possible that the small amount of ultraviolet could have any significant effect on the color of the area to the insect eye.

These areas of relatively high ultraviolet reflectance may be divided by their general color to human eyes, as follows:

1. *Color apparently wholly structural, i.e. highly iridescent:*

   Upper forewing green in *Callicore aurelia.*

   Upper forewing blue in *Morpho peleides.*

   Upper hindwing violet-blue in *Caligo illioneus.*

2. *Color partly structural:*

   a. A bluish iridescent film overlying a red spot or band of pigment:

      Upper hindwing margin in *Biblis hyperia.*

      Under forewing area in *Callicore aurelia.*

      Upper hindwing spot in *Papilio neophilus* (higher in ♀).

      Upper hindwing spot in *Papilio anchisiades* (♀).

   b. Structural scattering, refraction and reflection effects combined with yellow pigment:

      Upper wing creamy yellow in *Phoebis sennae* (♀).

      Under wing yellow in same species, both sexes, higher in ♀.

3. *Color apparently wholly pigmentary:*

   Upper wing green of *Victorina steneles.*

It will be seen from the above that in all except one species, *Victorina,* it is an area involving structural colors in which ultraviolet reflectance is relatively high. This species, however, also shows high reflectances in most of the remainder of the spectrum, putting it in the category of positively ultraviolet whites and pale tints, such as are commonly found on under wings where white is faintly washed with a color appearing in intense form on the upper surfaces (as in the pale pink underwing band of *Heliconius erato*). In yellow and creamy *Phoebis,* in which the color is partly structural (but non-iridescent) and

partly pigmentary, the values are much higher throughout the visible than in the ultraviolet, putting it in the usual pierid category of relatively negative ultraviolet whites and yellows.

Except in the Pieridae, white or pale tinted areas, whether wing spots, body spots, wing borders, bands or entire wings, reflect very strongly in the ultaviolet, as they do throughout the visible. In the pierids, on the other hand, ultraviolet reflectance is very low, especially in males, just as it is in white flowers (Richtmyer, 1923; Lutz, 1924; Lotmar, 1933).

The slight sexual differences found in the ultraviolet reflectances of some butterflies will be discussed below (p. 108).

Very few reflectance differences of appreciable extent emerge in the different regions of the ultraviolet. As explained on p. 95, this region was tested in three ways. First, with sunlight as the source, the photographs were made with a filter which, at the exposures used, transmitted only radiation shorter than 400 mμ; this was passed in a fairly broad arc with a maximum at 366 mμ. Second, the sun and this same filter were used in conjunction with an interference filter transmitting in a narrow band at 380 mμ. Third, a mercury vapor tube was used as a source, emitting in a narrow peak at 366 mμ, in conjunction with the usual filter to eliminate visible radiation. These three procedures gave very comparable results. In view of these checks, it seems highly unlikely that, in the species examined, there are any undiscovered bands of high ultraviolet reflectance wide enough to affect the color of the wing from the point of view of insect vision.

B. Reflectance in the Violet and Blue Regions

Most of the butterflies which are not white, pale yellow or pale green reflected very weakly in the violet and blue, the exceptions being the strongly iridescent areas of *Callicore, Morpho* and *Caligo*. In addition, the red spot of *Papilio neophilus,* particularly in the female, shows considerable reflectance in these regions.

C. Reflectance in the Blue-Green Region

Blue-green is a strong component in all butterfly colors tested except those appearing strongly orange, russet or red to the human eye; in these it is always extremely low or altogether negative. In contrast, it is strong in all yellows and their tints, in greens and in all strongly iridescent areas, although not in bluish iridescent films covering red pigments *(Callicore, Biblis, Papilio).*

D. Reflectance in the Green and Green-yellow Regions

Green and yellow-green are even more prevalent than the blue-green, being extremely weak or negative only in the purest reds, as in *Heliconius erato, H. ricini, Callicore aurelia, Anartia amalthea* and *Papilio* spp. In all the yellows, russets and oranges as well as in the strongly iridescent forms, these regions show moderate to high values, as well, of course, as in all whites and pale tints.

E. Reflectance in the Yellow Region

This region marks the beginning of the long-wave-region rise in all the reds mentioned in the preceding section except that of *Callicore,* which starts to rise only in the orange (600 mμ). For strongly yellow butterflies the maximum is not here, although reflectance is naturally high, but in the orange or orange-red, with reflectance in the green-yellow and green only slightly less than in the yellow. It is a strong component of orange and russet areas (*Dryas,* danaids, brown ithomiids, *Heliconius numata,* etc.) The iridescence of *Morpho* and *Caligo* shows a sharp falling off in the yellow.

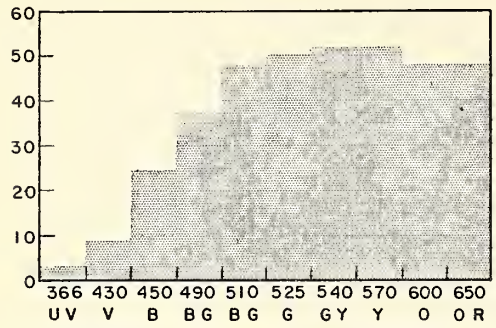
F. Reflectance in the Orange and Orange-red Regions

Practically all non-black areas in all butterflies tested show extremely high reflectance in the orange and orange-red. The exceptions are the iridescent blue and violet of *Morpho* and *Caligo.* In all the others, whether the area appears to human eyes as white, or as some form of green, yellow, orange, or red, the regions of the longest waves examined either equal or exceed those of the other regions. In areas appearing green, pale yellow-green or pale yellow the orange regions tends to be slightly higher in reflectance than the orange-red; in the purer yellows as in the orange and red areas, the orange-red component exceeds the orange in value.

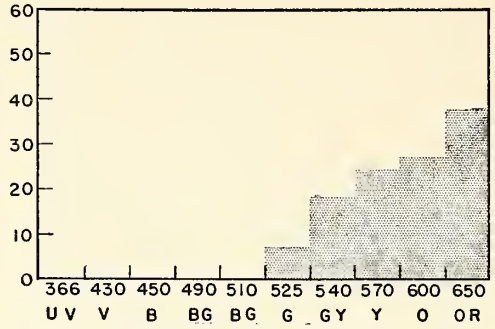
Discussion and Conclusions

The species analyzed in the preceding pages were selected on the basis of several criteria. It was desirable, first, to include varied examples of the most common local butterflies, second those with conspicuous small areas of possible signal or "recognition" value in social relationships, and finally illustrations of presumed mimicry. The group is not a systematically representative sample of Trinidad butterflies, since three families, the Lycaenidae, Erycinidae and Hesperidae, have been omitted. Nevertheless, it does comprise a generous sample of the color characteristics of typical, widely distributed neotropical species of representative colors and patterns, and so appears to furnish adequate data for a number of general observations.

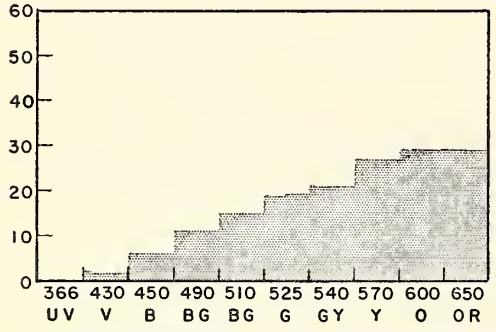
These remarks, however, are merely preliminary to analytical behavior studies on particular



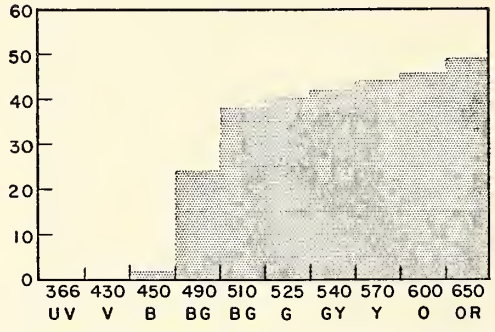
a. *Heliconius sara rhea* ♂: upper forewing, yellow spot.



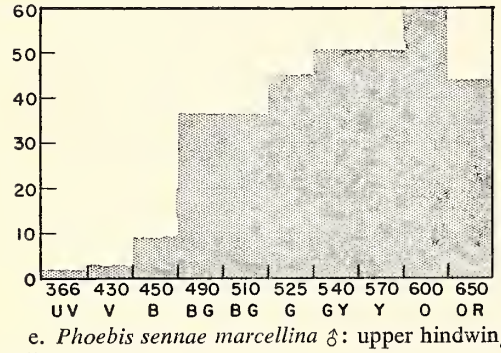
b. *Dryas julia julia* ♂: upper forewing, orange.



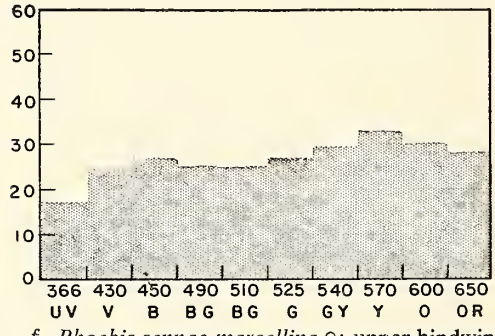
c. *Papilio thoas neacles* ♂: upper hindwing, yellow.



d. *Anteos maerula maerula* ♂: upper hindwing, yellow.



e. *Phoebis sennae marcellina* ♂: upper hindwing, yellow.



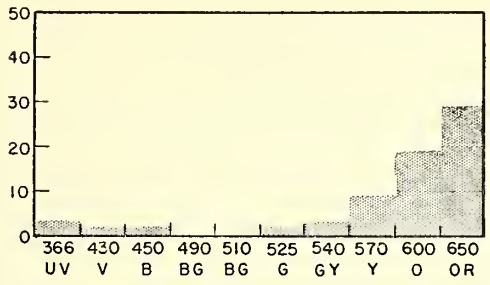
f. *Phoebis sennae marcellina* ♀: upper hindwing, yellowish.

TEXT-FIG. 8. Reflectance characteristics of areas of special interest in selected butterflies (cont.). Explanation as in Text-fig. 7.

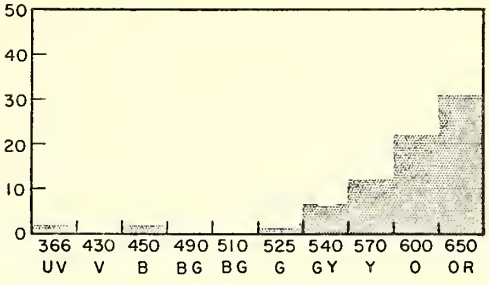
species of butterflies. Because of this, they include brief reference to experimental evidence not yet published as well as some speculative material. Both of these inclusions seem desirable in order to present an up-to-date general view of the field.

Possibilities of adaptive significance of ultraviolet reflectance in butterfly wings.—One of the primary objects of the present study was to determine whether ultraviolet reflectance from wings or body is likely to prove a widespread factor in the social behavior of the local butterflies. As has been said in the preceding section, the answer is decidedly in the negative. Excluding white and pale-tinted areas, only one-fifth of the butterflies examined had any areas reflecting more than 5% that of magnesium oxide at 366 mμ. In none of the remainder was significantly higher reflectance found between 366 and 400 mμ.

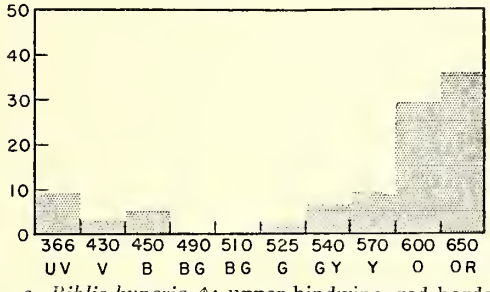
The majority, those reflecting less than 5% of ultraviolet, have such relatively strong reflectances in the visible that it does not seem possible that the low percentage of ultraviolet could, except by its subtractive effect, have any significant effect on the color of the area to the insect eye.



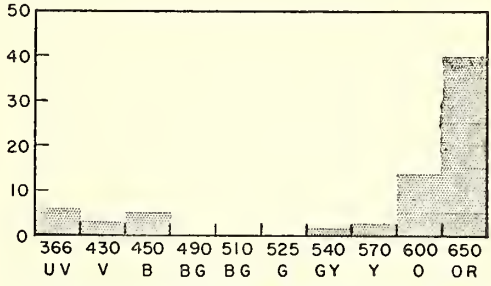
a. *Heliconius erato hydara* ♂: upper forewing, red band.



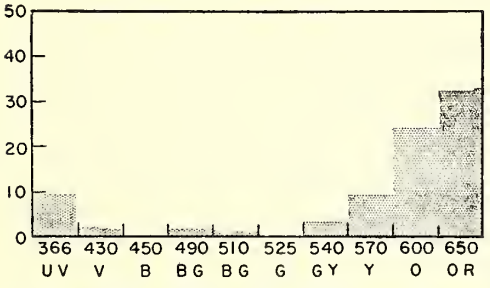
b. *Heliconius ricini insulana* ♂: upper hindwing, red area.



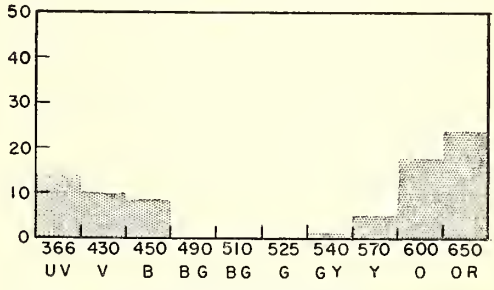
c. *Biblis hyperia* ♂: upper hindwing, red border. (Color partly structural).



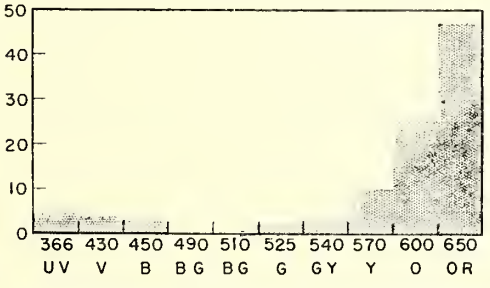
d. *Callicore aurelia* ♀: under forewing, red area. (Color partly structural).



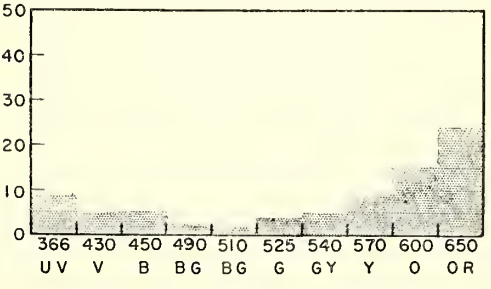
e. *Papilio neophilus parianus* ♂: upper hindwing, red spots. (Color partly structural).



f. *Papilio neophilus parianus* ♀: upper hindwing, red spots. (Color partly structural).



g. *Papilio anchises cymochles* ♀: upper hindwing, red spots. (Color partly structural).



h. *Papilio anchisiades anchisiades* ♀: upper hindwing, red spots. (Color partly structural).

TEXT-FIG. 9. Reflectance characteristics of areas of special interest in selected butterflies (cont.). Explanation as in Text-fig. 7.

This is especially true in view of the small amount of ultraviolet in sunlight reaching the earth under even ideal conditions (Text-fig. 3), which would tend to more than counter-balance the high responsiveness of insects generally to the ultraviolet (Text-fig. 2).

The question of the role played for butterflies by ultraviolet reflectance in areas appearing

white to human beings will now be considered. It seems certain that "white" for butterflies includes high reflectance in the ultraviolet between around 300 and 400 mμ, as well as in the visible, just as it does for bees (p. 89). The white areas in all families treated except the pierids reflect strongly in the ultraviolet. Therefore, they are undoubtedly "true" whites for the butterflies, since current experiments (in ms.) show first, that butterflies of all families included in this study are unquestionably highly sensitive to the ultraviolet, and, second, that at least in the Heliconiidae, negatively ultraviolet white is distinguished from positively ultraviolet white, and is treated by them as a color. It is also attractive to them under certain conditions, exactly as it is in bees. Also as in bees, positively ultraviolet white is never attractive or, apparently, particularly conspicuous to them. This may very likely be because of the prevalence of highly reflectant, unimportant surfaces, such as wet leaves, in their natural environment.

However that may be, it seems that ultraviolet reflectance from white wing areas can be of adaptive importance to butterfly social behavior in only two ways. The first would be as part of a distinctive pattern, such as in *Phyciodes, Dynamine* or *Adelpha.* Nothing is as yet known of this aspect. Second, in the pierids only, it might be significant by its absence. As has long been known, a low ultraviolet reflectance is a characteristic of the pterines which are largely responsible for the whites and yellows in this family (review in Timon-David, 1947). Ilse (1928; review 1941) found that *Pieris rapae* Linnaeus responded with courting behavior to yellowish-white paper models. At that time, however, she was not taking possible ultraviolet reflectance into consideration, it being then undetermined that pierids were sensitive to that region. If pierid color vision proves after all to be basically similar to that of bees and other butterflies, it would be a negatively ultraviolet white, an approach to bee-blue-green, to which they would respond in courting. However Ilse (1937; review 1941) has assembled strong indirect evidence that, for pierids, ultraviolet is not complementary to the blue-green-to-green region which they distinguish apart from blue and yellow (see p. 89). According to her experiments with egg-laying responses, "red, mauve and violet" are all treated as complementaries to green, which would seem to preclude ultraviolet in that role. The entire problem remains one of particular interest.

In 1952 Makino and his co-workers reported on the higher ultraviolet reflectance in female *Pieris rapae* compared with males, and gave the chemical characteristics responsible for the difference. These authors did not, however, report the degree of difference, or the amount of reflectance relative to a standard. In the present study, sexual differences in ultraviolet reflectance have also been found to be characteristic of all the pierid whites and yellows tested, as well as of the browns and russets of danaids and ithomiids. In all of these the ultraviolet reflectance is low —in the browns less than 4% on the upper sides in both sexes, and in the white and yellow pierids less than 5%, at least in the males. The greatest sexual difference was found in *Phoebis sennae,* in which the yellow male reflects about 2% and the pale, cream-colored female 17% or more from the upper wing surfaces. According to Hertz's experiments with bees (1937, 1939), it is necessary for a white paper to reflect more than a third in the ultraviolet of the level of the general reflectance in the visible in order to be treated as "white"—i.e., uncolored—by bees; if the relative reflectance in the ultraviolet is as low as one-fourth the level in the visible, it will be treated, in sunlight, as colored by bees—as equivalent, that is, to bee-blue-green. In the pierids, which are so highly reflectant in the visible, the increased reflectance in the ultraviolet appears to be rarely if ever enough to render the females uncolored to the males; presumably, however, the males would appear a purer hue than the females. Work on this problem remains to be done.

The pale green of *Victorina,* although it has rather high reflectance in the ultraviolet, reflects so strongly throughout most of the visible that it seems unlikely that the ultraviolet can affect its hue for butterflies except to render it more nearly white (uncolored).

The possibilities of adaptive significance of ultraviolet reflectances have now been considered and largely discounted in all except one small group. This includes those butterflies with iridescent areas on the wings (Table 4). These six species—a *Callicore,* a *Biblis,* a *Morpho,* a *Caligo* and two *Papilio*—alone of those analyzed seem likely to have evolved a connection between their ultraviolet reflectance and their social behavior. All of these systematically scattered forms have employed similar means, through structural colors (presumably of the interference type), of producing ultraviolet.

In the upper wings of *Morpho* and *Caligo* and the upper wing-bar of *Callicore,* interference colors are entirely responsible (excluding the usual dark pigmented bases to the laminated scale plates). In the remaining species, the under forewing of *Callicore,* the upper hindwing margin of *Biblis* and the upper hindwing spots of *Papilio,* a delicate, bluish iridescent film overlies a vivid red pigment. This film is invisible to

the human eye except at large angles of incident light.

In all of these iridescent effects, whether or not red pigment is also involved, the operation of the usual laws governing interference colors is very evident (see e.g., Richards, 1951, p. 197 ff., and Fox, 1953, p. 56 ff.). Briefly, and excluding variable side effects such as surface scattering of light by both fine and macroscopic irregularities, the smaller the angle of incidence, the longer the wavelengths of the region of highest intensity. As an example, a well-flattened small piece of *Morpho* wing, viewed directly in line with the light (that is, at a 0° angle) will appear blue-green to the human eye, at a 45° angle it will appear blue, and, finally at increased angles of incidence the highest relative ultraviolet values are recorded by photographic means.

However, in *Morpho* at least, because of the details of number, order, intervening distances and varying thickness of the superimposed plates, the highest absolute intensities occur when the wing is illuminated at the smaller angles of incidence (Anderson & Richards, 1942); at these angles ultraviolet values are at a minimum.

Now, the possible significance of these iridescent areas in intraspecific behavior may be as follows: With every wingbeat, a flying *Morpho* butterfly changes the angle of light incidence through the entire possible range. To the human eye, a *Morpho* in flight is simply a flickering flash of varying tints of blue. However, to another *Morpho,* in sunlight, there should be a brilliant shift from blue-green or blue to ultraviolet, then momentary extinction and back again through the spectral arc; conceivably this may be an exceptionally potent stimulus. The well-known dipping of these butterflies to blue papers and other objects suggests strongly that the wing color may prove to be a sign stimulus in inter-male or courtship behavior.

The delicate iridescent sheen of the red band of *Biblis,* and of the red spots of *Papilio* is invisible on the wing to human beings. Nevertheless, as in *Morpho,* it seems likely that these areas may prove to be of adaptive importance, the blue-to-ultraviolet effect, as in the strongly iridescent butterflies, being perhaps visible during flight, or when the wings are open and shut, in the characteristic motion of butterflies at rest. If so, it seems possible that the presumed aposematic coloring of *P. neophilus* and its mimic, *P. anchisiades,* may have a double function. The visibility of the iridescent red areas in both *Biblis* and *Papilio* may be strongly enhanced for insect vision by the breaking up of the red by means of the fine black cross-bars (see responses of butterflies and bees to form, Ilse, 1932.2; von Frisch, 1950).

The subdued iridescence of *Caligo illioneus* follows, in lower key, the same laws as the others, and to the insect eye the butterfly might well, in full sunlight, give a moderately strong blue-to-ultraviolet flash. However, this species flies only at twilight and only among trees, where ultraviolet is a negligible component of the feeble light. Therefore an adaptive use of its reflectance pattern in intraspecific behavior seems to be extremely unlikely in this species. Possibly the iridescence is here a vestigial character. Experimental work on these problems is in progress.

Reflectance in the Visible.—The major characteristic here is that the occurrence of reflectance is progressively higher toward the long wave end of the spectrum. Violet and blue are rare and scanty spectral components—except in structural colors where they are the rule. Blue-green is a strong component except in brown, russet, orange or red, while the various greens are weak or negative only in the orange-reds and reds. Yellow, orange and red are the most prevalent of all, being strong components of practically all butterfly pigments. In the present group, in fact, they are weak only in the spectra of the entirely structurally colored areas of *Morpho* and *Caligo.* This is altogether in accordance with the absorption spectra of various prevalent insect pigments, including carotenes, anthoxanthins and some pterins, in which violets and blues are largely absorbed (reviews in Timon-David, 1947; Karrer & Jucker, 1950; Fox, 1953).

An important point in connection with butterfly red is this, that all reds—whether in butterflies or in flowers—reflect highly in the orange as well, and often show also considerable amounts of yellow. Even if butterflies prove to be as weakly sensitive to wavelengths longer than 650 mμ as bees and other insects (p. 88, Text-figs. 1, 2), they would still be able to perceive with ease the yellow and orange components of the area— whether as one or more distinct hues is, at the moment, immaterial. Thus the well-known predilection of pierids and papilionids for red flowers in feeding (Ilse, 1928; Kaye, 1921, and present author's observations) may well be due to an attraction or extra sensitivity to the orange components, rather than to an extension of their spectral range at an efficient level into the red.

Results of Fading.—It is well known that the pigments of many butterflies fade considerably even in life; this is especially true locally of *Heliconius erato, Anartia amalthea* and *Victorina steneles.* In every case the results from the point of view of reflectance patterns are, for

the reds, higher reflectance (i.e., reduced absorption) in the orange and yellow. Similarly, in visually green areas, faded specimens show higher reflectance in the shorter wave regions, while the long wave reflectance is practically unchanged.

No significant difference in reflectance patterns was found between specimens freshly killed before photographing and those which had been dried, providing only that the specimens had been protected from light and rubbing. The use of chloroform or paradichlorobenzene had no observable effect on the reflectance results.

Conclusions.—The following statements, outside the realm of speculation, may now be made concerning the reflectance of the butterfly wing patterns analyzed, and in regard to the possible adaptive significance of the ultraviolet in intraspecific behavior.

1. In only five species out of 41 is ultraviolet reflectance at all likely to be involved adaptively in their social behavior. This is in the blue of *Morpho,* in the red hindwing border of *Biblis,* under forewing red of *Callicore* and the hindwing red spots of *Papilio neophilus* and *P. anchisiades,* both of the latter primarily in the female. In all of these species the colors are altogether or partly structural.

2. Except for the positively ultraviolet red areas mentioned, no unexpected areas of possible intraspecific signal or stimulating value were discovered. All blacks proved to be negatively ultraviolet; ocelli on the underwings of *Euptychia, Morpho* and *Caligo* were composed simply of minus-ultraviolet browns and russets and positively ultraviolet whites; neither cryptically colored areas, so prevalent on the underwings, nor the striking combinations of reds (except those previously noted), oranges, yellows, browns, blacks and whites of presumed aposematic coloring, and of Batesian or Mullerian mimicry, showed any unusual or unexpected spectral characteristics whatsoever. In other words, none of the reflectance patterns showed any hidden spectral components in the ultraviolet which might be interpreted as special adaptions to insect vision, rather than only to the vision of vertebrate enemies (see Cott, 1940; Goldschmidt, 1945; and Allee *et al.,* 1949, p. 669 ff., for recent reviews and comment on this still contentious subject).

3. All of this is in accordance with the conclusions of Lutz (1924) and Weiss (1945, 1946) on the apparent lack of correlation between the sensitivity of insects to the ultraviolet and the amount of ultraviolet either in their environment in general or reflected from the flowers which they pollinate.

In fact, it appears increasingly obvious that ultraviolet sensitivity is a mere byproduct of the physiological processes of the insect eye. The chemical steps in the formation and destruction of photosensitive pigments in insects have still to be worked out. Nevertheless, it is already known that the primary photosensitive substance in the insect retina has an absorption curve practically identical with that of rhodopsin in man (Roeder, 1953, pp. 515 ff. and ref. See also Prosser, 1950, pp. 408, 410-411, 422 ff. and ref. for related subjects). However, unlike the vertebrate eye, in which ultraviolet is largely absorbed before reaching the retina, this short-wave region is unimpeded by the outer elements of the insect ommatidium.

In brief, it appears from the present study that only rarely has any correlated evolution of wing color occurred in response to this ultraviolet sensitivity which could be of possible significance in intraspecific relations, and that in the few potential examples, the ultraviolet role is played by structural rather than pigmentary colors.

4. The high reflectance in the orange of all red butterfly pigments would theoretically make these "reds" of usable brightness to the insects, even though they prove to have, as do other orders of insects, negligible visual sensitivity around and above 650 mμ.

Conclusions on the adaptive value of color and pattern in the visible, and of the juxtaposition of hues, in intraspecific behavior must await the completion of forthcoming studies.

Summary

1. A method is described of determining the spectral composition of the colors of butterfly wings. The technique employs photography through combinations of narrow band pass and interference filters with peak transmissions ranging from 366 to 650 mμ. A scale of standards, of known spectrophotometric reflectance in terms of magnesium oxide, is included in each negative. Negative images are subsequently analyzed with a densitometer, their densities being compared with the standards in each frame. By this means preliminary analyses of entire wings were carried out, as well as detailed examinations of highly magnified areas of special interest.

2. Forty-one species of Trinidad butterflies belonging to 28 genera were thus analyzed as a prerequisite to studies of the adaptive value of color, including the ultraviolet, in intraspecific behavior.

3. Only eight of these species show any areas other than white and pale tints having more than 5% reflectance in the ultraviolet. The colors of

all of these areas are either partly or altogether structural in nature. In only five of these species is it considered at all likely that these special areas, all more or less iridescent, would prove to be of adaptive value in intraspecific behavior.

4. Spectral characteristics in the visible are unsurprising, with a preponderance of reflectance in the long wave regions, regardless of apparent hue. Examples of presumed Batesian and Mullerian mimicry show similar spectral patterns, all with minimal ultraviolet reflectances. It is pointed out that the high reflectance of all reds in the orange region would make these pigments of adequate visibility even to insects for which the visible spectrum may be curtailed beyond the orange-red.

References

Allee, W. C., A. E. Emerson, O. Park, T. Park & K. P. Schmidt

1949. Principles of Animal Ecology. Philadelphia & London: W. B. Saunders Co., xii + 837 pp.

Anderson, T. F. & A. G. Richards, Jr.

1942. An electron-microscope study of some structural colors of insects. Jour. Appl. Physics, 13: 748-758.

Anon.

1945. Wratten light filters. 17th Ed., Revised. Eastman Kodak Co. 86 pp.

Beebe, W.

1952. Introduction to the ecology of the Arima Valley, Trinidad, B.W.I. Zoologica, 37: 158-184.

Bertholf, L. M.

1931. The stimulative efficiency in the ultraviolet spectrum for the honey bee. Jour. Agric. Res., 43(8): 703-713.

1932. The extent of the spectrum for *Drosophila* and the distribution of stimulative efficiency in it. Zeitschr. Vergl. Physiol., 18: 32-64.

Brues, C. T.

1941. Photographic evidence on the visibility of color patterns in butterflies to the human and insect eye. Proc. Amer. Acad. Arts & Sci., 74(8): 281-285.

Cockayne, E. A.

1924. The distribution of fluorescent pigments in the Lepidoptera. Trans. Entom. Soc. London, 1924: 1-19.

Coste, F. H. P.

1890-1891. Contributions to the chemistry of insect colours. Entomologist, 23: 128 *et al.*; 24: 9 *et al.*

Cott, H. B.

1940. Adaptive coloration in animals. New York. Oxford University Press, ix + 508 pp.

Crane, J., & H. Fleming

1953. Construction and operation of butterfly insectaries in the tropics. Zoologica, 38: 161-172.

Crescitelli, F. & T. L. Jahn

1939. The electrical response of the dark-adapted grasshopper eye to various intensities of illumination and to different qualities of light. Jour. Cell. and Comp. Physiol., 13: 105-112.

Ford, E. B.

1941-1947. Studies on the chemistry of pigments in the Lepidoptera, with reference to their bearing on systematics. Proc. R. Ent. Soc. London, 1941 (A) 16: 65-90; 1942, 17: 87-92; 1944.1, 19: 92-106; 1944.2, 19: 201-223; 1947.1, 22: 72-76; 1947.2, 22: 77-78.

Fox, D. L.

1953. Animal Biochromes and Structural Colours. Cambridge University Press, xiv + 378 pp.

Frisch, K. von

1915. Der farbensinn und formensinn der bienen. Zool. Jahrb. Abt. Allg. Zool. und Physiol., 35: 1-182.

1948. Aus dem leben der bienen, 4th edition. Wien: Springer-Verlag. 196 pp.

1950. Bees: their vision, chemical senses, and language. Cornell Univ. Press, Ithaca, N. Y. xiii + 119 pp.

Gentil, K.

1942. Elektronmikroskopische untersuchung des feinbaues schillernder leisten von morphoschuppen. Zeitschr. Morph. Ökol. Tiere, 38(2): 344-355.

1943. Zum morphologie und optik der schillerschuppen von *Phyllobius argentatus* L. (Col.). Senckenbergiana, 26: 244-251.

Goldschmidt, R. B.

1945. Mimetic polymorphism, a controversial chapter of Darwinism. Quart. Rev. Biol., 20: 147-164, 205-230.

Graham, C. H., & H. K. Hartline

1935. The response of single visual sense cells to lights of different wavelengths. Jour. Gen. Physiol., 18(16): 917-931.

Harrison, C. R., R. C. Lord, & J. R. Loofbourow

1948. Practical spectroscopy. Prentice-Hall, New York. xiv + 605 pp.

Hertz, M.

1937.1 Beitrag zum farbensinn und formensinn der bienen. Zeitschr. Vergl. Physiol., 24: 413-421.

1937.2 Versuche über das farbensystem der bienen. Die Naturwiss., 25: 492-493.

1937.3 Zur technik und methode der bienenve suche mit farbpapieren und glasfiltern. Zeitschr. Vergl. Physiol., 25: 239-250.

1939. New experiments on color vision in bees. Jour. Exper. Biol., 16: 1-8.

Hopkins, F. G.

1895. The pigments of the Pieridae: a contribution to the study of excretory substances which function in ornament. Trans. Roy. Soc. Lond., (B) 186: 661-682.

Ilse, D.

1928. Uber den farbensinn der tagfalter. Zeitschr. Vergl. Physiol., 8: 658-692.

1932.1 Eine neue methode zur bestimmung der subjektiven helligkeitswerte von pigmenten. Biologisches Zentralblatt, 52: 660-667.

1932.2 Zur "formwahrnehmung" der tagfalter. I. Spontane bevorzugung von formmerkmalen durch vanessen. Zeitschr. Vergl. Physiol., 17: 537-556.

1937. New observations on responses to colors in egg-laying butterflies. Nature, 140: 544.

1941. The color vision of insects. Proc. Roy. Soc. Glasgow, 65: 68-82.

Jahn, T. L. & F. Crescitelli

1939. The electrical responses of the Cecropia moth eye. Jour. Cell. and Comp. Physiol., 13: 113-119.

Jahn, T. L. & V. J. Wulff

1948. The spectral sensitivity of *Dytiscus fasciventris*. Jour. New York Ent. Soc., 56(2): 109-117.

Karrer, P., & E. Jucker (Trans. & Rev. by Braude, E. A.)

1950. Carotenoids. New York & Amsterdam: Elsevier Publ. Co., Inc.; London: Cleaver-Hume Press, Ltd., x + 384 pp.

Kaye, W. J.

1921. A Catalogue of the Trinidad Lepidoptera Rhopalocera (Butterflies). Mem. Dept. of Agriculture, Trinidad and Tobago, No. 2, xii + 163 pp.

Knoll, F.

1926. Insekten und blumen. H. 1-6. Abh. Zool.-Bot. Ges. Wien, 12 (1921-1926).

Köhler, P.

1926. Los pigmentos alares. Rev. Soc. Ent. Argentina, 1(2): 45-49.

Kühn, A.

1927. über den farbensinn der bienen. Zeitschr. Vergl. Physiol., 5: 762-800.

1946. Konstructionsprinzipien von Schmetterlingsschuppen nach electronenmikroskopischen aufnahmen. Zeitschr. für Naturf., 1: 348-357.

Kühn, A., & M. An (von Engelhardt)

1946. Elektronenoptische untersuchungen über den bau von schmetterlingsschuppen. Biol. Zbl., 65: 30-40.

Lotmar, R.

1933. Neue untersuchungen über den farbensinn der bienen, mit besonderer berucksichtigung des ultravioletts. Zeitschr. Vergl. Physiol., 19: 673-724.

Lubbock, J.

1882. (Revised ed. 1932). Ants, bees and wasps. Appleton, New York.

Lutz, F. E.

1924. Apparently non-selective characters and combinations of characters, including a study of ultraviolet in relation to the flower-visiting habits of insects. Ann. N. Y. Acad. Sci., 29: 181-283.

1933.1 "Invisible" colors of flowers and butterflies. Journ. Amer. Mus. Nat. Hist. (New York), 33(6): 565-576.

1933.2 Experiments with "stingless bees" (*Trigona cressoni parastigma*) concerning their ability to distinguish ultraviolet patterns. Amer. Mus. Novitates No. 641.

Makino, K., K. Satoh, M. Koike, & N. Ueno

1952. Sex in *Pieris rapae* L. and the pteridin content of their wings. Nature, No. 4335, Nov. 29, pp. 933-934.

Mason, C. W.

1926. Structural colors in insects, I. Jour. Phys. Chem., 30: 383-395.

1927. Structural colors in insects, II. Jour Phys. Chem., 31: 321-354.

Mayer, A. G.

1896. The development of the wing scales and their pigments in butterflies and moths. Bull. Mus. Comp. Zool. Harvard, 29: 209-236.

1897. On the colour and colour-patterns of moths and butterflies. Bull. Mus. Comp. Zool. Harvard, 30: 169-259.

Mayer, F., & A. H. Cook

1943. The chemistry of natural coloring matter. Reinhold, N. Y.

Moon, P.

1940. Proposed standard solar-radiation curves for engineering use. Jour. Franklin Inst., 1940: 583-617.

Passos, C. F. dos

1948. Occurence of anthoxanthins in the wing pigments of some nearctic *Oeneis*. Ent. News, 59(4): 92-96.

Prosser, C. L. (Editor)

1950. Comparative animal physiology. W. B. Saunders Co., Philadelphia. ix+888 pp.

Richards, A. G.

1951. The integument of arthropods. Univ. of Minnesota Press, Minneapolis. xvi+411 pp.

Richtmyer, F. K.

1923. The reflectance of ultraviolet by flowers. Jour. Optical Soc. Amer. & R. S. I., 7: 151-168.

Ridgway, R.

1912. Color standards and color nomenclature. Publ. by the author. Washington, D. C.

Roeder, K. D. (Editor)

1953. Insect physiology. John Wiley & Co., N. Y., Chapman & Hall, Ltd., London. xiv+ 1100 pp.

Schlieper, C.

1927. Farbensinn der tiere und optomotorische reaktionen. Zeitschr. Vergl. Physiol., 6.

Schmidt, W. J.

1942. Über die farbung der flügelmembran bei *Papilio teredon* Fldr. und *Papilio agamemnon* L. Zeitschr. Morph. ökol. Tiere, 38(2): 334-343.

Suffert, F.

1924. Morphologie und optik der schmetterlingsschuppen ins besondere die schillerfarben der schmetterlinge. Zeitschr. Morph. ökol. der Tiere, 1: 171-308.

Thompson, D. L.

1926. The pigments of butterflies' wings. Biochem. Jour., 20: 73-75, 1026-1027.

Timon-David, J.

1947. Pigments des insectes. l'Année Biol., 23: 237-271.

Weiss, H. B.

1943.1 The group behavior of 1400 insects to colors. Ent. News, 54(7): 152-156.

1943.2 Color perception in insects. Jour. Econ. Ent., 36: 1-17.

1944. Insect responses to colors. Jour. N. Y. Entom. Soc., 52: 267-271.

1945. Insect response to colors. Scientific Monthly, July, 1945, Vol. LXI, 51-56.

1946. Insects and the spectrum. Jour. N. Y. Entom. Soc., 54: 17-30.

Weiss, H. B., F. A. Soraci & E. E. McCoy, Jr.

1941. Notes on the behavior of certain insects to different wave-lengths of light. Jour. N. Y. Ent. Soc., 49: 1-20; 149-159.

1942. The behavior of certain insects to various wave-lengths of light. Jour. N. Y. Ent. Soc., 50: 1-35.

Wigglesworth, V. B.

1924. Uric acid in the Pieridae: a quantitative study. Proc. Roy. Soc., B, 97: 149-155.

1946. Insect physiology. Methuen's Monographs on Biological Subjects. London. Third Ed. viii + 134 pp.

1949. Insect biochemistry. Ann. Rev. Biochem., 18: 595-614.

Wolf, E.

1942. Spacial relations of ommatidia in insects and differential sensitivity to moving visual stimuli. Anat. Rec., 84(4): 469-470.

EXPLANATION OF THE PLATES

Plate I

Species and subspecies of butterflies used in color analyses. Not all the specimens appearing in this plate were captured in Trinidad; therefore size ratios are not always typical of the island populations. All species, or their close relatives, are illustrated in color in Seitz: Macrolepidoptera of the World; The American Rhopalocera, Vol. V, Plates. (1924).

Fig. 1. *Danaus plexippus megalippe.*
Fig. 2. *Lycorea ceres ceres.*
Fig. 3. *Tithorea mopsa megara.*
Fig. 4. *Mechanitis doryssus veritabilis.*
Fig. 5. *Hypothyris euclea euclea.*
Fig. 6. *Hypoleria ocalea.*
Fig. 7. *Ithomia drymo pellucida.*
Fig. 8. *Hymenitis andromica trifenestra.*
Fig. 9. *Euptychia hermes hermes.*
Fig. 10. *Euptychia hesione.*
Fig. 11. *Heliconius numata ethilla.*
Fig. 12. *Heliconius erato hydara.*
Fig. 13. *Heliconius sara rhea.*
Fig. 14. *Heliconius ricini insulana.*
Fig. 15. *Heliconius aliphera aliphera.*
Fig. 16. *Dryas julia julia.*
Fig. 17. *Agraulis vanillae vanillae.*
Fig. 18. *Phyciodes ofella ofella.*
Fig. 19. *Phyciodes leucodesma.*
Fig. 20. *Anartia amalthea amalthea.*
Fig. 21. *Victorina steneles steneles.*
Fig. 22. *Biblis hyperia.*
Fig. 23. *Callicore aurelia.*
Fig. 24. *Colobura dirce dirce.*
Fig. 25. *Dynamine theseus.*
Fig. 26. *Dynamine artemesia.*
Fig. 27. *Adelpha iphicla daceleia.*
Fig. 28. *Adelpha cytherea insularis.*
Fig. 29. *Protogonius hippona trinitatis.*
Fig. 30. *Morpho peleides insularis.*
Fig. 31. *Caligo illioneus saltus.*
Fig. 32. *Papilio anchises cymochles* ♀.
Fig. 33. *Papilio neophilus parianus* ♂.
Fig. 34. *Papilio neophilus parianus* ♀.
Fig. 35. *Papilio thoas neacles.*
Fig. 36. *Papilio anchisiades anchisiades* ♂.
Fig. 37. *Anteos maerula maerula.*
Fig. 38. *Phoebis sennae marcellina.*
Fig. 39. *Eurema albula* f. *albula.*
Fig. 40. *Eurema venusta.*
Fig. 41. *Melete lycimnia harti.*

Plate II

Preliminary surveys of spectral characteristics of butterflies: Sample negative film strips photographed through only six filter combinations. Entire butterflies are shown, along with knife blade coated with magnesium oxide and used as a rough standard. Each film strip includes, from left to right, frames covering roughly the six following regions: ultraviolet (peak transmission at 366 mμ), violet-blue (peak at 430 mμ), blue-green (peak at 510 mμ), green-yellow (peak at 540 mμ), yellow (peak at 570 mμ), orange-red (peak at 640 ff.). For complete explanation, see text, p. 91 ff.

Fig. 42. In each frame, from left to right: *Callicore aurelia,* right under side; *Adelpha iphicla daceleia,* upper; *A. cytherea insularis,* upper.

Fig. 43. In each frame, upper sides: left, *Heliconius numata ethilla;* right, *Tithorea mopsa megara.*

Fig. 44. In each frame, from left to right, upper sides: *Heliconius aliphera aliphera; Agraulis vanillae vanillae; Papilio neophilus parianus* ♀. (Positions of frames 11 and 12 are transposed in the reproduction since, in exposing these two negatives, the respective filter combinations were inadvertently used in transposed order).

Plate III

Fig. 45. Negative illustrating arrangement of butterfly wings and standards in photography by sunlight with ultraviolet filter. The four vertical pairs of images in central portion of negative are the white wings of a pierid, *Eurema albula,* arranged as follows from left to right; under wings, ♀; upper wings, ♀; under wings, ♂; upper wings, ♂. The 19 steps of gray standards are placed, in two strips, above and below wings; colored standards of high ultraviolet reflectance are in vertical strip near right edge of negative. Note minimum ultraviolet reflectance of upper wings in ♂. The unevenness of density in images of individual wings is a typical result of sunlight photography of colors due partly to structure rather than almost entirely to pigment, and illustrate

the care needed in analysis with the densitometer; see p. 96.

FIG. 46. Negative illustrating arrangement of small pieces of butterfly wings and standards in photography by sunlight with ultraviolet and interference filters, giving a narrow peak transmission at 380 mμ. The six central images are pieces cut from the red pigmented forewing bands of individual *Heliconius erato hydara,* representing both sexes and various ages. Gray standards placed as in Fig. 45; colored standards near left edge of negative.

FIG. 47. Negative illustrating arrangement of wing pieces and standards in photography indoors by ultraviolet lamp and ultraviolet filter. Five central images, from left to right, *Papilio thoas neacles,* upper hindwing yellow with black margins (practically negatively ultraviolet and therefore invisible in reproduction); *Heliconius sara rhea,* upper forewing, yellow spot surrounded by black; *Heliconius ricini insulana,* upper forewing, yellow spot surrounded by black; *Eurema venusta,* ♀, under forewing, yellow; same, under hindwing, whitish. (See Figs. 48-50 for same group photographed through other filter combinations). Colored and gray standards (darker half) in two strips, above and below images, respectively. Note low reflectance, indicated by low negative density, of all specimens except *Eurema.*

FIG. 48. Same specimens, photographed by photoflood lamp through filters with a peak transmission in the violet-blue (430 mμ). Only darker half of gray standards is included.

FIG. 49. Same specimens, photographed by photoflood lamp through filters with a peak transmission in the orange (600 mμ). Only lighter half of gray standards is included. Note high reflectance indicated by high negative density, of all specimens here and in Fig. 50.

FIG. 50. Same specimens, photographed by photoflood lamp through filters with a peak transmission in the orange-red (650 mμ). Only lighter half of gray standards is included.

FIG. 51. Red hindwing patches of *Papilio* spp., photographed by photoflood lamp through filters with a peak transmission in the orange (600 mμ). Only lighter half of gray standards is included. From left to right: *Papilio anchisiades anchisiades,* ♂; same, ♀; *P. anchises cymochles,* ♂ (piece of spot only); *P. neophilus parianus,* ♂; *P. anchises cymochles,* ♀.

FIG. 52. Same, photographed through filters with a peak transmission in the orange-red (650 mμ).

FIG. 53. Arrangement of camera, stand and lights for indoor photographic analysis of butterfly colors.

SPECTRAL REFLECTANCE CHARACTERISTICS OF BUTTERFLIES
(LEPIDOPTERA) FROM TRINIDAD, B.W.I.

FIG. 42

FIG. 43

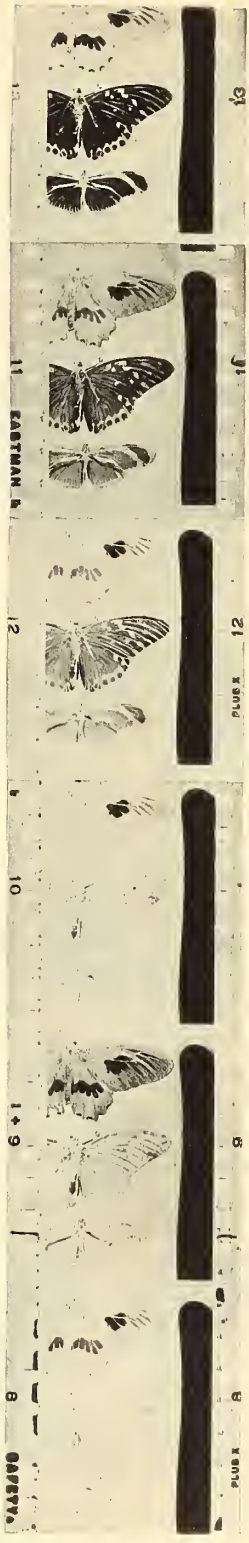


FIG. 44

SPECTRAL REFLECTANCE CHARACTERISTICS OF BUTTERFLIES
(LEPIDOPTERA) FROM TRINIDAD, B.W.I.

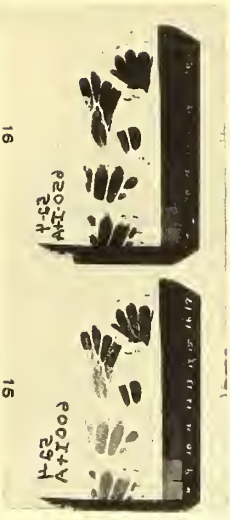


FIG. 51

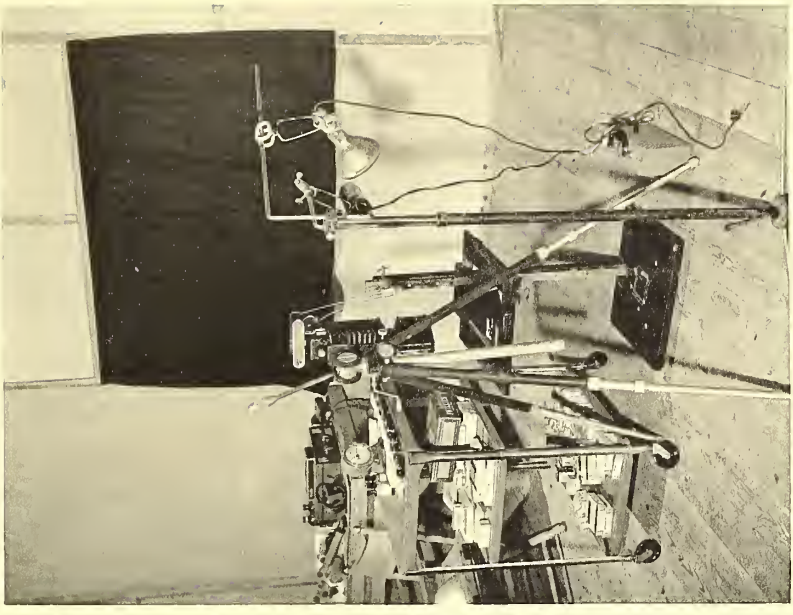


FIG. 52

FIG. 53

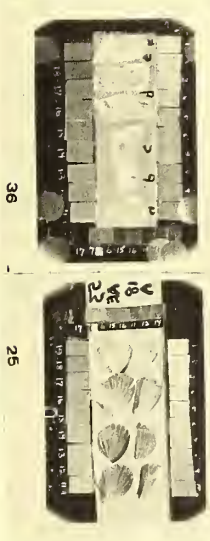


FIG. 45

FIG. 46

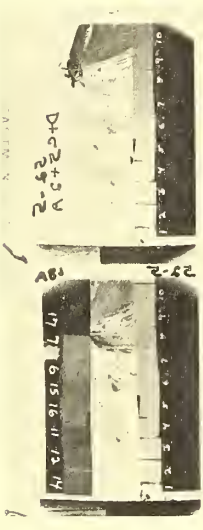


FIG. 47

FIG. 48

FIG. 49

FIG. 50

SPECTRAL REFLECTANCE CHARACTERISTICS OF BUTTERFLIES
(LEPIDOPTERA) FROM TRINIDAD, B.W.I.

9

Failure to Elicit the Galli-Mainini Reaction in *Rana pipiens* with Spawning Reflex Fractions and Other Teleostean Pituitary Preparations, and Observations on the Response to Mammalian Gonadotrophins

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Introduction

THE ROLE of male Salientia in human pregnancy diagnosis has received much attention in the medical and clinical literature since its introduction by Galli-Mainini (1947). The test is based on the observation that chorionic gonadotrophin, which is usually present in high concentrations in the urine of women in the first trimester of pregnancy, will cause the release of spermatozoa (the gametokinetic response) in tailless amphibians. Since this response can be induced in frogs in which the anterior lobe of the pituitary has been removed, it is concluded that the mammalian gonadotrophic hormones act directly upon the test animal's gonadal tissue (Kissen, 1954).

The South American toad, *Bufo arenarum,* was the first species to be utilized in pregnancy testing, but since then many different frogs and toads from all over the world have been found suitable. In North America, Wiltberger & Miller (1948) found the readily available and easily maintained leopard frog, *Rana pipiens,* to be both sensitive and reliable. The conditions under which this species may be utilized have been studied by many investigators. During the summer months the leopard frog is least sensitive (Holyoke & Hoag, 1951; Pollak, 1950; Reinhart *et al.,* 1951). Giltz & Miller (1950) showed that leopard frogs kept at cool temperatures and in shallow water were more sensitive than those stored similarly at room temperatures; the optimum temperature for the response, according to these authors, ranges from 15° to 22° C., and reactivity was reported to decrease above and below this. Soucy (1949) found that the reactivity of *R. pipiens* is not affected by keeping these frogs in darkness. The investigations of Haskins & Sherman (1949, 1952) showed that the time of appearance of spermatozoa in the frog's urine is inversely proportional to the amount of chorionic gonadotrophin administered and that this relationship provides a fairly accurate method of bio-assay. Similarly, using pregnancy urine in various dilutions, Marsters *et al.* (1950) found that the intensity as well as the time of the response was related to the hormone titer.

Male amphibians have also been reported to react positively to the pituitary gonadotrophins of mammals (see review by Houssay, 1949). Robbins (1951) and Robbins & Parker (1952) found that follicle stimulating hormone (FSH) caused the release of spermatozoa in *Rana pipiens,* and Hobson (1952) reported that male *Xenopus laevis* are very sensitive to this hormone. On the other hand, Thorborg & Hansen (1951) found that *Bufo bufo* gave no response to FSH in concentrations equivalent to eight Mouse Units. Crézé (1949) reported that the luteinizing hormone (LH) caused the discharge of sperm in *Rana esculenta.* Both FSH and LH gave positive reactions in the Indian toad, *Bufo melanostictus* (Bhaduri, 1951). According to Houssay (1949), *Bufo arenarum,* reacted positively to toad, rat and human hypophysis. Greenblatt, Clark & West (1949) noted that prolactin evoked the gametokinetic reaction in *Rana pipiens,* but Houssay, Penhos & Burgos (1953) found that *Bufo arenarum* did not react to prolactin. No other anterior pituitary hormones have been reported to give a positive gametokinetic response in male Salientia (Robbins & Parker, 1952; Houssay, 1952) and, at the time of writing, the situation in respect to the two gonadotrophins (FSH, LH) and prolactin requires further clarification. Adrenalin is known to elicit this response in frogs but not in toads (Robbins & Parker, 1949; Hinglais & Hinglais, 1949, 1953); however, an error resulting from

this is unlikely under ordinary circumstances and may be discounted.

The present investigation is part of an analysis of the pituitary hormones of fishes undertaken by one of us (G. E. P.) in collaboration with Dr. Alfred E. Wilhelmi of Emory University. It was found (Pickford, 1952) that crude extracts of fish pituitary, as well as certain purified fractions contained a factor that elicited the spawning reflex in both normal and hypophysectomized killifish (*Fundulus heteroclitus*). The problem arose as to whether this factor could be identified with either of the well known mammalian gonadotrophins. Mammalian follicle stimulating hormone (FSH) was found to be inactive, as was also prolactin, but the luteinizing hormone (LH) elicited a moderately strong response. For this reason it was thought that the spawning reflex factor might be identical with LH. The Galli-Mainini test appeared to provide a sensitive and reliable method by which this hypothesis could be tested.

Acknowledgements

The experiments reported here were conducted in the laboratory of the New York Aquarium at the New York Zoological Park. Facilities for this purpose were generously provided by Curator and Aquarist Christopher W. Coates, to whom our best thanks are extended. The investigation was sponsored by a grant from the Mearl Corporation of New York City, through kindness of Mr. Harry E. Mattin and Dr. Leon M. Greenstein. We should like to take this opportunity of acknowledging their generous support and continued interest in the progress of research in this field of investigation. The fish pituitary glands were collected at Wilson's Beach, Campobello Island, N. B., where Mr. William Jackson most kindly provided facilities for this purpose. The purified fractions were prepared by Dr. Alfred E. Wilhelmi of the Department of Biochemistry, Emory University. Our best thanks are also due Dr. Sanford L. Steelman of Armour and Co., and to the Schering Corporation for the donation of some of the mammalian preparations. Chloromycetin was donated by Parke Davis and Co. We are indebted to Professor Alexander Petrunkevitch of Yale University for a translation of the article by Stroganov & Alpatov (1951) and we wish to thank him for his generous help in our search of the Russian literature.

Materials and Methods

Male frogs, *Rana pipiens,* averaging 30 grms. in weight, were procured from Mr. Walter Daniels, Mt. Ephraim, New Jersey. The animals were housed in a dark, unheated room. The temperature approximated that of the outdoors, and ranged from 5° to 12° C. The well known seasonal variation in the sensitivity of *Rana pipiens* to gonadotrophins cannot be a factor in the present study since the experiments were conducted in winter months, from December, 1953, to middle of March. The frogs were housed in 15-gallon glass aquaria, 10 to 18 animals per tank, with about an inch of tap water covering the slate bottom. Since the animals were not fed, it was sufficient to clean the tanks every five or six days, at which time the frogs were also washed in cold running tap water.

Although most of the animals were in excellent condition, a few showed early symptoms of red leg disease, i.e., a slight reddening of the lower abdomen and legs. Such animals were isolated and treated with chloromycetin in the water, as recommended by Ambrus *et al.* (1951). As a prophylactic measure, chloromycetin was also added to the stock tanks. The incidence of the infection was greatly reduced by this procedure, but the disease was not wholly eradicated.

A few hours before running a test, the heat was turned on in the laboratory, and the temperature raised to 15° to 22° C. This is the optimum range for the gametokinetic reaction (Giltz & Miller, 1950). Frogs to be used for the tests were first examined for the presence or absence of spermatozoa by inserting into the cloaca a thin glass pipette containing a little water. A few drops of cloacal fluid were removed to a glass slide and studied under the microscope. No cases of spontaneous emission were encountered. Test frogs were kept singly in plastic containers, about five inches square with perforated lids; about 2 cc. of water were placed in the bottom of each container. At least two frogs were employed for every test. Animals that gave a positive reaction were kept separately and were not used again for at least one week.

Injections were made into the dorsal lymph sac, using a half-inch, 25-gauge needle. The preparations to be injected were made up in physiological saline (0.7% sodium chloride); concentrations were varied but not the volume injected, which was 1 cc. per frog. The mammalian chorionic gonadotrophin, Antuitrin S (Parke Davis Co.), was used as a standard of reference. Under the conditions of our experiments it was found that 10 I. U., corresponding to 0.04 mgms. of the preparation used (Lot No. M718M), was the minimum dose that would elicit a response within one hour. With this

concentration, occasional or small numbers (up to ca. 75) of motile spermatozoa were seen in most fields under the low power. This order of sensitivity is higher than that reported by Haskins & Sherman (1949), but is of the same order as that found by Holyoke & Hoag (1951). For the purpose of screening the preparations to be tested, a high dosage level was selected, viz. 4 mgms. per cc. per injection; this corresponds to the maximum dosage level that had been used on *Fundulus heteroclitus* in screening the same preparations for spawning reflex activity, viz. 100 microgrs. per gram weight. It is to be noted that this dose is 100 times stronger than the minimum dose of Antuitrin S that was just sufficient to elicit a consistent response. In some cases multiples or fractions of the standard dose were also tested. In testing unknown preparations, two hours were allowed to elapse before the urine was examined.

The following mammalian pituitary preparations were tested: prolactin (Schering Corp., Batch 4g. Hyex 4), luteinizing hormone (Armour Co.), follicle stimulating hormone (Armour Co.), and three preparations submitted by Dr. Wilhelmi. The LH was known to have spawning reflex activity in *Fundulus,* while the prolactin and FSH were inactive in this respect (Pickford, 1952). Two of Dr. Wilhelmi's preparations (B88C and B88E) also had spawning reflex activity, but the third was inactive (P88F). We are indebted to Dr. Sanford L. Steelman for the following additional information regarding the two Armour preparations: "Sheep LH, Lot No. 227-80: This is a purified LH preparation which is about 80 to 85% homogeneous, electrophoretically and ultracentrifugally. It contains traces of FSH and TSH. . . . Swine FSH, Lot No. K45208R: This preparation is a purified FSH which can be briefly characterized by Step II FSH described in Steelman *et al.,* 1953." The last mentioned reference is of particular interest since one may deduce from it that the FSH contained LH activity of the order of three to four percent. The significance of this will appear below.

The fish pituitaries were collected in the summer of 1950 and again in the summer of 1952, from three species of Gadidae: the Boston hake, *Urophycis tenuis* (Mitchill), the cod, *Gadus morhua* Linnaeus, and the pollock, *Pollachius virens* (Linnaeus). Details of the procedure have been described by Pickford (1954). The 1950 batch consisted mostly of pollock, the 1952 batch mostly of hake. This difference has a possible significance, apart from problems of species specificity, since the hake had already spawned whereas the cod and pollock were in pre-spawning condition. The three species were mixed in the bulk material that was frozen and shipped to Dr. Wilhelmi, but a limited number of glands from each species were dropped into acetone, and pulverized powders derived from this material were used in some of the experiments in an attempt to determine whether there were differences between the hake and the other two species. In addition, hake glands were frozen in small packets of ten glands each, and a brei prepared from this material was used in one series of frog tests. Dr. Wilhelmi prepared twelve separate fractions from the 1950 material, all of which were tested on hypophysectomized killifish for anterior lobe activities (Pickford, unpublished). One fraction was toxic, probably owing to the presence of zinc (F7A), four gave evidence of ACTH activity (F4A, F4B, F4C and F5A), three had spawning reflex activity (F6D, F6EF and F6G), one was the fish growth hormone (F6B), and two were inactive (F6C, F31A). Six fractions from the 1952 material were also tested; these had not been subjected to the complete screening survey, as described above, but two of them had moderately strong spawning reflex activity (F80D and F80x), whereas the other four showed little or no activity (Pickford, unpublished). All the fish pituitary preparations, crude or purified, contained a melanophore concentrating agent that induced pallor in killfish (but not in frogs).

Results

(a) *Mammalian Pituitary Preparations.*—Armour's LH was found to have about the same order of activity as Antuitrin S, i.e., a weakly positive response could usually be elicited at a concentration of 0.04 mgms. per cc. Concentrations of 0.1 mgms. per cc. and higher always elicited a vigorous response. Both these preparations were between 40 and 100 times more active than Armour's FSH. The latter preparation was inactive at 2 mgms. per cc. or less, but induced a weak to moderate response at 4 mgms. per cc. The results can be interpreted if it is assumed that the response is elicited by LH but not by FSH, since the FSH preparation used was known to contain three to four percent LH. This order of contamination would account very closely for our results. It would be necessary to use an even more highly purified FSH preparation to determine whether or not this gonadotrophin of itself can evoke the gametokinetic response.

Prolactin was inactive, as also were the three mammalian preparations submitted by Dr. Wilhelmi.

(b) *Fish Pituitary Preparations.*—Of the 20 different fish pituitary preparations that were tested, only the brei of frozen hake glands gave any indications of activity. The response was of a very low order: 3 out of 7 frogs that received an injection of two hake glands per cc. (approximately equivalent to 4 mgms. of dry acetone powder) showed a weak to moderate reaction, the other 4 frogs being negative. Moreover, negative results were obtained at both higher and lower dosage levels: 12 glands (3 frogs), 6 glands (3 frogs), and 0.5 gland (2 frogs). Extracts of an acetone powder of hake glands were completely negative both at 12 and 4 mgms. per cc. (2 frogs each). To make perfectly sure that an occasional positive response might not be obtained, the test on the acetone powder of hake glands was repeated at the 4 mgm. per cc. dosage level, using 10 frogs. The results were again negative. Negative results were also obtained with acetone powders of pollock (12 mgms. per cc. on 2 frogs) and cod (12 mgms. per cc. on 2 frogs; 4 mgms. per cc. on 2 frogs). This finding eliminates the possibility that a seasonal inactivity of the hake glands was involved, resulting from their post-spawning condition.

In view of the findings reported above with crude extracts, it is not surprising that all of the purified or partially purified fish pituitary fractions that were tested also gave negative results. Whatever may be the cause of the weak response that was obtained from the brei of frozen glands, it is clear that there is no correlation with the spawning reflex activity of the preparations.

Discussion

Three separate problems are involved in this investigation: (1) the question as to which mammalian anterior lobe hormone can elicit the Galli-Mainini response, (2) the question as to whether the spawning reflex factor, present in both mammalian and fish pituitary preparations, can be identified with any of the known anterior lobe hormones, and (3) the problem of the reactivity of amphibians to gonadotrophins derived from fishes.

In regard to the first problem, our results suggest that only LH can elicit the response. Previous reports of positive responses with FSH or prolactin can probably be attributed to traces of contamination with LH, as appears to have been the case in the FSH sample which was studied by us.

In regard to the second problem, it is perfectly clear that the spawning reflex factor is not the same as the pituitary hormone, presumably LH, that elicits sperm release in male frogs.

In regard to the third problem, some further discussion is desirable since the negative results obtained by us conflict with other work that has been reported in the literature. Little work has been done on the effect of fish pituitaries on amphibians, and most of it concerns the effect on ovulation in the female frog or toad. Implantation into the dorsal lymph sac of either small or large numbers of pituitaries from the teleosts *Salvelinus namaycush, Perca flavescens* or *Stizostedion vitreum* (Creaser & Gorbman, 1936) and the elasmobranch *Squalus suckleyi* (Creaser & Gorbman, 1939) evoked no ovulatory response in *Rana pipiens.* Negative results were also obtained in female *Bufo arenarum* after repeated pituitary implants (Houssay *et al.,* 1929) and after the injection of saline extracts of glands from an unidentified species of fish (Houssay & Giusti, 1930). Similarly, ovulation was not induced in a species of *Rana* following the injection of extracts of glands from *Gadus merlangus* (Rostand, 1934). On the other hand, Wills, Riley & Stubbs (1933) obtained ovulation in the toad, *Bufo americanus,* after one to five daily injections of two to four pituitary glands of the gar, *Lepisosteus platysomus.* These authors also reported ovulation in *Rana pipiens* after three daily injections of four glands from the same species of fish.

Stroganov & Alpatov (1951) appear to be the only workers who have tested fish pituitaries using the gametokinetic response of male frogs. They obtained positive results within one hour in *Rana temporaria* and *R. ridibunda* using concentrations of 0.15 to 0.8 mgms. of acetone-dried pituitaries of the Russian sturgeon, *Acipenser güldenstädti.* Unspecified concentrations of this extract also evoked a positive response in *Rana esculenta.* These authors found that frogs were approximately three times more sensitive to injections of sturgeon pituitary than was the loach, *Misgurnus fossilis.*

The results of Stroganov & Alpatov stand in striking constrast to ours. Neither suspensions of acetone powders, at higher dosage levels than those employed by the Russian workers, nor extracts of a variety of purified or partially purified fish pituitary fractions, gave positive results with *Rana pipiens.* A weak and uncertain response was obtained even with an extract of whole frozen hake glands. Our experiments were made with pituitaries of the relatively specialized marine teleosts belonging to the family Gadidae, whereas Stroganov & Alpatov employed sturgeon glands. Creaser & Gorbman (1939) pointed out that the gars, *Lepisosteus,* are phylogenetically closer to the Amphibia than any of the other fishes whose pituitaries

had been used up to that time; the same relatively close relationship exists between the sturgeons and the Amphibia. It is obvious that phylogeny as well as species specificity may be involved.

That Stroganov & Alpatov found frogs to be more sensitive than fish for the assay of fish pituitary gonadotrophins, is also of interest in the light of the views of Creaser & Gorbman (1936, 1939). After extensive experimentation, these investigators concluded that interspecific difference in the gonadotrophic hormones was the factor responsible for the inactivity of fish gonadotrophins on female *R. pipiens*. This, they maintained, also made it necessary to use relatively high concentrations of mammalian hormones to evoke ovulation in amphibians, while homeo-transplants or implants were effective in much lower concentrations. However, our male frogs were very sensitive both to mammalian chorionic gonadotrophin and to LH. It is not possible to state whether or not hormone specificity is in part responsible for the negative results which we obtained with fish pituitary preparations. Even if hormone specificities are involved, however, there seems to be no close correlation with phylogenetic relationships.

Summary

1. Under the conditions of our experiments, the minimum dose of chorionic gonadotrophin (Antuitrin S, Parke Davis) that would elicit sperm release in *Rana pipiens* in one hour was 10 I. U. (= 0.04 mgms.). Armour's LH (80 to 85% homogeneous) was active at the same dosage level. The activity of a preparation of Armour's FSH was proportional to its LH content (three to four percent). Prolactin (Schering) was inactive.

2. A brei of frozen pituitary glands from the hake (*Urophycis tenuis*) occasionally elicited sperm release at a dosage level of two glands per frog (= ca. 4 mgms. dry weight).

3. Neither extracts of acetone-dried powders of hake, cod (*Gadus morhua*) or pollock (*Pollachius virens*), nor a variety of pituitary fractions derived from these species (A. E. Wilhelmi) showed any activity at doses of 4 mgms. or higher.

4. Neither fish nor beef pituitary fractions that were known to have spawning reflex activity when tested on the killifish, *Fundulus heteroclitus*, had any effect on male frogs.

5. It is concluded that the spawning reflex factor is not the same as the mammalian luteinizing hormone (LH).

6. The conflicting literature on the response of amphibians to fish pituitary gonadotrophins is discussed.

Literature Cited

Ambrus, J. L., C. M. Ambrus & J. W. E. Harrison

1951. Prevention of *Proteus hydrophilus* infection (red leg disease) in frog colonies. Amer. J. Pharm., 123: 129.

Bhaduri, J. L.

1951. The role of Salientia in human and mammalian pregnancy tests. Proc. 38th Indian Sci. Congress, Bangalore. Sect. Zool. and Entomol.: 1-30.

Creaser, C. W. & A. Gorbman

1936. Specificity of the pituitary gonadotropic factor as demonstrated in amphibia. Copeia, 1936: 91-94.

1939. Species specificity of the gonadotropic factors in vertebrates. Quart. Rev. Biol., 14: 311-331.

Crézé, J.

1949. Sensibilité du testicule des batraciens à l'hormone lutéinisante hypophysaire (LH). Compt. Rend. Soc. Biol., 143: 1331-1332.

Galli-Mainini, C.

1947. Pregnancy test using the male toad. J. Clin. Endocrinol., 7: 653-658.

Giltz, M. L. & D. F. Miller

1950. Ways of improving the male frog test for pregnancy. Ohio J. Sci., 50: 205-209.

Greenblatt, R. B., S. Clark & R. M. West

1949. Hormonal factors producing the gametokinetic response in the male frog (Rana pipiens). J. Clin. Endocrinol., 9: 668.

Haskins, A. L., Jr. & A. I. Sherman

1949. Quantitative bio-assay of chorionic gonadotrophin with the male frog. Endocrinology, 44: 542-545.

1952. The quantitative assay of serum chorionic gonadotrophin in pregnancy, using the modified male frog technique. J. Clin. Endocrinol., 12: 385-392.

Hinglais, H. & M. Hinglais

1949. Actions de l'adrenaline sur l'excrétion spermatique chez le grenouilles mâles. Compt. Rend. Soc. Biol., 143: 811-812.

1953. Nouvelles recherches sur l'action des venin de crapauds sur la spermato-migration provoquée chez la grenouille. Compt. Rend. Soc. Biol., 147: 1924-1925.

Hobson, B. M.

1952. Conditions modifying the release of spermatozoa in male *Xenopus laevis* in response to chorionic gonadotrophin. Quart. J. Exper. Physiol., 37: 191-203.

Holyoke, J. B. & E. E. Hoag

1951. Seasonal variations in the pregnancy test using *Rana pipiens*. Amer. J. Clin. Pathol., 21: 1121-1126.

Houssay, B. A.

1949. Hypophyseal functions in the toad Bufo arenarum Hensel. Quart. Rev. Biol., 24: 1-27.

1952. Action des hormones hypophysaires de mammifère chez le crapaud et les grenouilles. Compt. Rend. Soc. Biol., 146: 137-139.

Houssay, B. A. & L. Giusti

1930. Fonction sexuelle, hypophyse et hypothalamus chez le crapaud. Compt. Rend. Soc. Biol., 104: 1030-1031.

Houssay, B. A., L. Giusti & J. M. Lascano-Gonzalez

1929. Implantation d'hypophyse et stimulation des glandes et des fonctions sexuelles du crapaud. Compt. Rend. Soc. Biol., 102: 864-866.

Houssay, B. A., J. C. Penhos & M. H. Burgos

1953. Modificaciones de la espermiacion producida por gonadotrofinas en el sapo. Rev. Soc. Argentina Biol., 29: 108-117.

Kissen, A. T.

1954. Effect of gonadotropic hormones on hypophysectomized (anterior lobe) male *Rana pipiens*. Ohio J. Sci., 54: 187-191.

Marsters, R. W., M. E. Black & J. D. Randall

1950. An evaluation of the Rana pipiens male frog pregnancy test. Amer. J. Obstet. Gynecol., 60: 752-762.

Pickford, G. E.

1952. Induction of a spawning reflex in hypophysectomized killifish. Nature, 170: 807.

1954. The response of hypophysectomized male killifish to purified fish growth hormone, as compared with the response to purified beef growth hormone. Endocrinology, (in press).

Pollak, O. J.

1950. Limited usefulness of male amphibia for pregnancy tests. J. Lab. Clin. Med., 36: 127-129.

Reinhart, H. L., I. J. Caplan & G. Y. Shinowara

1951. Factors influencing the male frog test for pregnancy. Amer. J. Clin. Pathol., 21: 624-634.

Robbins, S. L.

1951. Observations on the use of the male North American frog (Rana pipiens) in pregnancy diagnosis. J. Clin. Endocrinol., 11: 213-220.

Robbins, S. L. & F. Parker

1949. The reaction of male frogs to epinephrine. Endocrinology, 44: 384-388.

1952. Studies on the mechanism of the spermatic release of the male North American frog (Rana pipiens). J. Clin. Endocrinol., 12: 354-360.

Rostand, J.

1934. Hypophyse et ovulation chez les batraciens. Compt. Rend. Soc. Biol., 117: 1079-1081.

Soucy, L. B.

1949. The use of ordinary toads and frogs for pregnancy tests. Amer. J. Med. Technology, 15: 184-196.

Steelman, S. L.; W. A. Lamont, W. A. Dittman & E. J. Hawrylewicz

1953. Fractionation of the swine follicle stimulating hormone. Proc. Soc. Exper. Biol. Med., 82: 645-647.

Stroganov, N. S. & V. V. Alpatov

1951. "A new unit for determining the activity of the hypophysis in fish." Rybnoe Khoziaistvo, 27: 59-60. [In Russian].

Thorborg, J. V. & K. Hansen

1951. The use of Xenopus laevis, Bufo bufo, and Rana esculenta as test animals for gonadotrophic hormones. III. Quantitative investigations on the sensitivity of the animals to chorionic gonadotrophin. Acta Endocrinol., 6: 51-66.

Wills, I. A., G. M. Riley & E. M. Stubbs

1933. Further experiments on the induction of ovulation in toads. Proc. Soc. Exper. Biol. Med., 30: 784-786.

Wiltberger, P. B. & D. F. Miller

1948. The male frog, *Rana pipiens*, as a new test animal for early pregnancy. Science, 107:198.

10

Transplantation of the *Sc* Melanoma in Fishes

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(Plates I-V; Text-figures 1-4)

MELANOMAS have been described in representatives of all major groups of vertebrate animals, but successful transplantations of these black neoplasms have been reported only in mammals and amphibians. We now report the successful implantation and growth of a melanoma in fishes when the tumorous fragments are placed in the host's integumentary tissues between the scales. Previously, attempts to implant fish melanomas into the eye or peritoneum had failed but some success had been attained in an intermuscular site.

We (Marcus & Gordon, 1953) found that the genetic constitution of the host was of paramount importance in determining the success or failure of the growth of transplanted tumor tissue. This is in line with results obtained with other experimental animals. In the transfer of melanoma cells in fishes, autotransplantations were most successful. Transplantation to animals closely related to the host were successful less frequently. No success was attained when tumor fragments were placed in genetically unrelated fishes.

The transplantation of melanoma fragments into the transparent integumentary tissues between the scales of fishes has permitted us to observe the progressive transformations of the tumor cells in the host. The events were traced under a compound microscope by a method somewhat similar to the one developed by Algire (1943), who utilized a transparent window in his study of the growth of melanomas in mice.

It was hoped that continuous observations of individual tumor cells would give us the ontogenetic history of the melanocytes, the primary cell type of the definitive melanoma. While the evidence we have obtained is still incomplete, it appears that some of the melanocytes transform into macromelanophores. These large pigment cells have been determined by genetic experiments of Gordon (1951) to be the initiating elements for the development of melanomas in the members of certain genetic stocks of fishes.

Material and Methods

The fish melanomas successfully transplanted had originated spontaneously from a series of matings between Montezuma swordtails (*Xiphophorus montezumae*) carrying the dominant *Sc* gene, and common swordtails (*Xiphophorus helleri*), recessive for the same gene. The *Sc* gene in the normal *montezumae* is expressed by the presence of large black pigment cells or macromelanophores on the caudal fin. In *montezumae* × *helleri* hybrids of the first generation, the hypertrophic growth of macromelanophores produced much larger pigmented areas. By backcrossing the black-spotted hybrids to *helleri* swordtails, fish were obtained in which the caudal areas were intensely pigmented by macromelanophores. Atypical pigment cell growth led to the development of definitive melanomas at the site of abnormal pigmentation in the tail area, (Pl. I, Fig. 1; Pl. V).

In a preliminary statement, Gordon & Nigrelli (1949) described the *Sc* melanoma in *montezumae* × *helleri* hybrids, in part, as follows: "The corium is completely replaced by proliferating macromelanophores, which vary in size, shape and amount of melanin present, both within the same tumor and among similar tumors in different fish. The melanin-bearing cells at the periphery of the growth appear to be larger, show more numerous dendritic

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processes and are more loosely arranged than those towards the center of the tumor mass. There is considerable infiltration and destruction of muscle, bone and cartilage by these melanin-bearing cells. Numerous macrophages are invariably present at the periphery of the growth, with an occasional one containing melanin. In some regions of the growth the epithelium is thin and broken, probably as a result of the expanded corial growth. However, the epithelium in the region of the fins is often appreciably thickened. The tumor is especially characterized by extensive development of capillaries and sinuses together with numerous lacunae throughout, but especially at the periphery of the growth."

Hybrid fish bearing melanomas were placed for a few minutes in an anaesthetic solution of 1:3,000 of Sandoz' MS-222 (Tricaine methanesulfonate) to which the disinfectant Merthiolate (Lilly), 1:10,000, was added. Pieces of the melanoma were cut from the donor and teased apart in sterile "amphibian" saline (0.68% NaCl). Tumor fragments (0.2 to 0.8 mm) were inserted into the host as follows: A scale was raised slightly and then the untrimmed tumor particle was inserted by means of a blunt glass needle into the deepest part of the scale pocket in a region free of macromelanphores, far anterior to the caudal peduncle. No visible hemorrhages occurred during or following the operation. Some unimplanted tumor fragments were preserved in fixing fluids.

The black tissue implants were visible, owing to the transparency of the epidermis and scales, and were observed and photographed or drawn with the aid of the high power of a compound microscope. After suitable intervals some host animals with transplanted tumors were fixed in Bouin's and selected areas were sectioned. The slides were stained either with Masson's variant of Mallory's trichrome stain or with haematoxylin and eosin.

In the experiments and observations reported here, the names of cells follow the nomenclature recommended at the Third Conference on the Biology of Normal and Atypical Pigment Cell Growth (Gordon, 1953, and Fitzpatrick & Lerner, 1953):

Melanoblast: an embryonic cell potentially capable of producing melanin.

Melanocyte: a mature melanin-producing and melanin-containing cell.

Macrophage: a cell containing phagocytized melanin.

Melanophore: a pigment effector cell in lower vertebrate animals.

Types and Histories of Transplants

(Tables 1 and 2, Pls. I and II)

Autotransplants.—An *Sc* melanoma fragment was implanted into an anterior, non-macromelanophore-bearing area of the skin of the same animal. Fate: Six of nine transplants developed successfully. Some host animals with their actively growing implants were fixed after

Table 1. Transplants of Spotted Caudal (*Sc*) Melanoma

Number	Donor	Host	Fate	Remarks
			9 Autotransplants	
3	h42*Sc*	Same	Temporary	Resorbed in 2 to 6 weeks
6	h42*Sc*	Same	Permanent	Some fixed after 8 months, others after 24
			9 Homotransplants Series "A"*	
6	h42*Sc*	h42*Sc*	Temporary	Persistent up to 5 weeks
3	h42*Sc*	h42*Sc*	Permanent	One degenerated after 2 months, then grew again
			21 Homotransplants Series "B"*	
3	h42*Sc*	h42*sc*	Temporary	Resorbed in 4 to 5 weeks
4	h42*Sc*	h42*sc*	Negative	
5	h50*Sc*	h50*sc*	Temporary	Resorbed in 3 to 6 weeks
9	h50*Sc*	h50*sc*	Negative	
			18 Heterotransplants	
7	h50*Sc*	h42*Sc*	Negative	
1	h50*Sc*	h42*Sc*	Temporary	Resorbed after 2 weeks
3	h42*Sc*	182	Negative	Host = *X. helleri* albino
4	h50*Sc*	287	Negative	Host = Spotted-belly *X. helleri* × *X. maculatus* hybrid
3	h50*Sc*	281	Negative	Host = *X. maculatus*

*In homotransplants "A" donors and hosts were dominant for the *Sc* gene for macromelanophores on the caudal fin. In series "B" the hosts were recessive for the *sc* gene and did not have macromelanophores.

TABLE 2. FATE OF *Sc* MELANOMA TRANSPLANTS

	Auto-transplants	Homotransplants* Series "A"	Homotransplants* Series "B"	Hetero-transplants	Totals
Number Implants	9	9	21	18	57
Successful "Takes"	6	3	0	0	9
Non-persisting Cell outgrowths	3	6	8	1	18
Failures (No activity)	0	0	13	17	30

* In homotransplants "A" the donors and hosts were dominant for the *Sc* gene for macromelanophores on the caudal fin. In series "B" the hosts were recessive for the *sc* gene and did not have macromelanophores.

eight, others at 24 months. The rates of growth of four autotransplants are given in Table 3.

Homotransplants.—A fragment of an *Sc* melanoma was removed from one tumorous fish and implanted into two kinds of its siblings. In series "A" both donor and host carried the dominant *Sc* gene for *spotted caudal* pattern. In series "B" the host was recessive for this factor. Fate: In group "A," three of nine transplants persisted as long as the host lived, which was eight to 24 months. In group "B" none of the transplants was successful. In both groups several transplants grew for a short period, three to six weeks, and then regressed and disappeared.

Heterotransplants.—In this group, the fishes providing the tumor tissue differed more widely in their genetic constitutions from the host animals. Some transfers of the *Sc* melanomas were made from tumorous hybrids to members of the succeeding generations from brother to sister matings; others were made to members of the backcross generation. Some hosts represented different species. Fate: All but one of 18 heterotransplants failed to make any growth at all; one grew for two weeks, then disappeared.

MICROSCOPIC OBSERVATIONS OF IMPLANTS *in situ* (Pl. III)

Immediately after a successful implantation, the tumorous fragment becomes surrounded by a light gray halo of free melanin granules. Within five to 36 hours, the free melanin granules are phagocytized by macrophages; they appear as black clumps. Within about one week most melanophages migrate to the edge of the scale and disappear. Eventually the area of the implant is cleared of pigment cell debris.

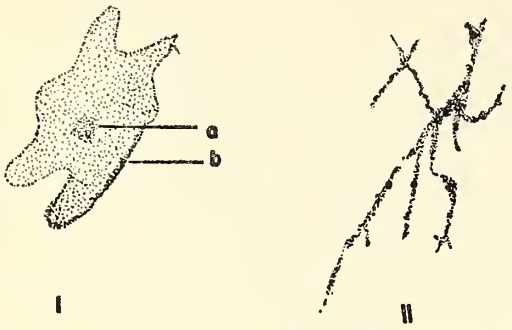
On the third to sixth day, dendritic processes of pigment cells emerge from the margin of the implant. Some of these are long and slender with pointed or clubbed tips, others are broad and sheet-like with short, spinous extensions.

After the sixth day, two kinds of melanocytes emerge from the implant. The smaller type (Text-fig. 1; Pl. IV, Fig. 1) has a few, thin pseudopodial processes, containing irregularly scattered melanin granules; it resembles the "small melanoblast" described by Grand & Cameron (1948) from tissue-cultures of fish melanomas. The larger type of melanocyte (Text-fig. 1; Pl. IV, Figs. 2 & 3) has a wide cell body, broad pseudopodial processes, and contains evenly distributed melanin granules; it resembles the cell that Grand & Cameron (1948) called a "large melanoblast." We believe it is a juvenile macromelanophore because we have observed the successive steps in the maturation of a similar cell (Text-fig. 2; Pl. IV, Figs. 3 & 4). When first seen, the young macromelanophore was elongate, broad, with-

TABLE 3. GROWTH OF SEVEN *Sc* MELANOMA IMPLANTS DURING TWENTY-FOUR MONTHS

Size in Millimeters of Implants by Months

Initial	2	4	6	8	24
		Autotransplants			
0.6	1.4	1.9	2.3	2.4	Fixed
0.4	1.7	2.0	2.1	2.2	Fixed
0.5	2.0	3.0	3.9	4.1	4.6
0.2	0.5	0.6	0.8	1.0	Fixed
		Homotransplants			
0.7	2.5	3.6	4.2	5.1	5.3
0.7	1.9	3.2	3.3	3.3	Fixed
0.3	0.5	0.7	1.2	1.2	1.4



TEXT-FIG. 1. Diagrammatic representation of a large (I) and a small (II) melanocyte (cf. Pl. IV, Figs. 1 to 4). The large melanocyte is characterized by the accumulation of pigment at the center of the cell (a) and at the periphery (b). The small melanocyte is highly dendritic and appears darker. (About 50×.)

out pseudopodial processes, and contained evenly distributed melanin granules. After four days the cell was more discoid and had definite pseudopodial processes. The processes contained evenly distributed melanin granules, while the center of the cell had an increased amount of melanin. After six days the large melanocyte looked like a typical melanophore.

An apparent reversal was also observed; that is, a melanophore had changed into a melanocyte. When first seen the melanophore had many short, slender, melanin-containing pseudopodial processes. Four days later, the melanin granules were more evenly distributed throughout the cell. Its slender pseudopodial processes disappeared; it was now rounder and its processes quite short and broad. Six days later, its cell outline was still more rounded and then it had the characteristics of a typical melanocyte. Between the fourth and sixth day it had moved a distance of 30 micra.

The melanocytes possess considerable migratory activity; the melanophores do not. Text-figure 3 shows three melanocytes and a record of their movements: one cell (b) moved away from the implant while the other two cells (a, c) moved parallel to its margin. Usually, the cells became sedentary after three to six days.

In order to distinguish the true pigment cells (that is, melanocytes and melanophores) from macrophages with their phagocytized melanin (melanophages), a small drop of a 1:1,000 solution of trypan blue in "amphibian" saline (0.68% NaCl) was placed under a slightly raised scale in a lightly pigmented portion of the skin. The dye filled the scale pocket and formed a diffuse blue area immediately beneath the scale. Within twenty-four hours, the particles of dye began to come together in a manner similar to the clumping of melanin granules around the freshly implanted melanoma fragment. Clumps of trypan blue particles, apparently within macrophages, passed to the edge of the scale and disappeared. No melanocytes, typified by their dendritic processes, picked up the color, nor did mature melanophores.

HISTOLOGY OF TRANSPLANTS IN HOST TISSUE (Pl. IV, Figs. 5-7)

Cross-sections through the transplants revealed black tissues in the dermis so densely pigmented by hypertrophied macromelanophores that no cellular structures were definable, a condition that typifies melanosis. Only along the margin of the transplant were pigment cell processes seen. There was no obvious vascular response in the host's tissues that were in contact with the implant.

The invasive pigment cells spread along the ventral surfaces of the scales and along the external muscle fascia below the dermis. The pigment cells eventually invaded the central portions of the dermis of the host, and occasionally occupied the entire dermal area. The subcutaneous muscles of the host were rarely and only slightly involved.

The histology of the transplant differed from

TEXT-FIG. 2. Transformation of a large melanocyte into a macromelanophore as traced by study of a single cell, located at the edge of a growing autotransplant, and drawn at 48-hour intervals. When first seen (I), the cell had the characteristics of a large melanocyte. Later (II), it developed pseudopodial processes, attained more melanin pigment and rounded up somewhat (cf. Text-fig. 1). Still later (III), the same cell attained the morphological characteristics of a melanophore. (From camera lucida drawings, about 50×.)

that of the melanoma fragment originally inserted. The high vascularity, blood-filled lacunae and the many unpigmented or slightly pigmented melanocytes characteristic of the *Sc* melanoma were wanting in the persistent transplant. After eight or even after 24 months of activity, the implant assumed the histological characteristics of a melanosis, a condition produced by the hypertrophic growth of macromelanophores. This kind of abnormal growth of large pigment cells is found in spotted xiphophorin fish hybrids prior to the development of melanoma.

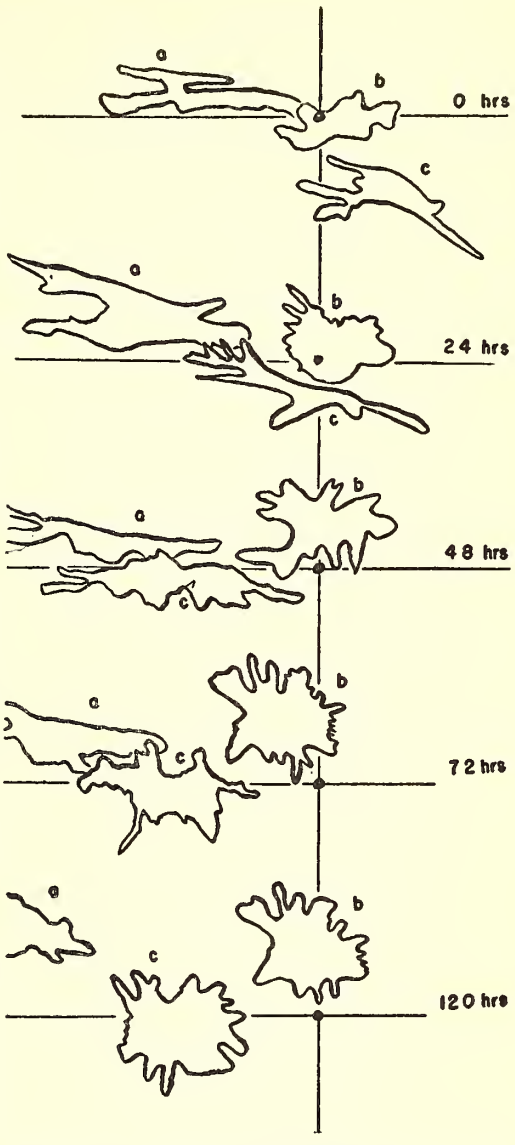
Discussion

Gordon & Smith (1938), Grand, Gordon & Cameron (1941) and Levine (1948) have drawn attention to the morphological similarity between melanoma cells of xiphophorin fish and mammals. There are also physiological similarities between mammalian and piscine melanomas. For example, Algire & Legallais (1948) called attention to the minimal vascular reaction around the growth of implanted melanomas in mice. Similarly, there is no apparent vascular reaction in the skin of the fish after implantation of a melanoma fragment.

The growth characteristics of the transplantable melanotic tumors in axolotls, as described by Sheremetieva-Brunst & Brunst (1948) resemble those of fishes. In the early stages the invading cells of one amphibian tumor travel along paths established by connective tissues. From the corium, the melanophores break through the subcutaneous fascial tissue and penetrate the musculature. Not all melanotic tumors have the ability to penetrate the subcutaneous tissues, but they occupy most of the corial tissues.

With regard to the influence of genetic correspondence between the donor and host tissues for successful growth of transplantable melanomas, the situation in mice is revealing. The Harding-Passey mouse melanoma, according to Algire & Legallais (1948), may be transplanted to mice of many strains, but the Cloudman S-91 melanoma is successful only in members of the *dba* strain. In amphibia, Krontovsky (1916) reported that not even autotransplants of melanomas were successful. Those studied by the Brunsts were successful when introduced into axolotls of apparently different strains. Their success, however, may have resulted from better transplantation techniques.

Melanomas from hybrid fishes failed to grow when transplanted into the peritoneums or eyes of other strains, but some success was obtained with a tumor transfer into the superficial muscles of an albino swordtail (Pl. II, Fig. 2). In contrast, even with the better technique, no success

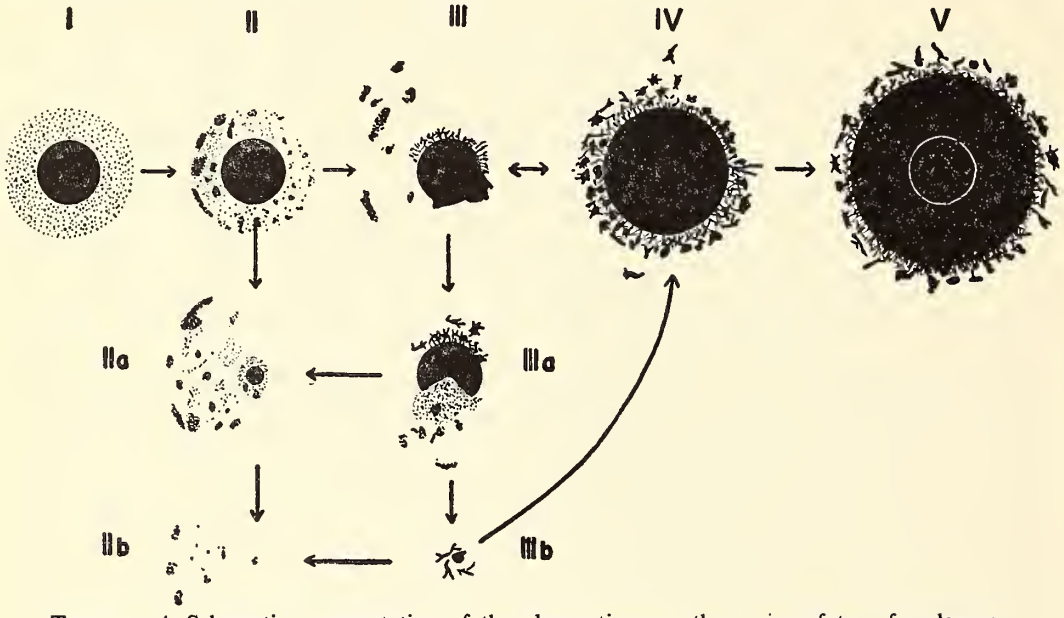


Text-fig. 3. Migration of melanocytes as traced by studying three melanocytes at the margin of a growing homotransplant. Melanocyte *a* travelled 170 microns in 120 hrs.; then, it could no longer be followed. Melanocyte *b* had a considerable accumulation of pigment, both near its center and around its edges (although this is not shown); it migrated hardly at all and after 72 hours it transformed into a melanophore; after this, it remained stationary. Melanocyte *c* migrated about 75 microns during the first 48 hours; during this time pigment developed at its center and at its periphery; then, at 72 hours, the cell stopped migrating and transformed into a melanophore. (Camera lucida, about 50×.)

was obtained in the present series when melanoma particles from hybrids were placed between the scales of the swordtail. This may possibly be explained by the fact that in the present heterotransplants swordtails of a highly inbred strain were used, whereas previously they were not. Text-fig. 4 shows a schematic representation of the results obtained in the present study.

Goodrich & Nichols (1933) transplanted, into unpigmented areas of the skin of goldfish, scales with their adhering dermal tissues containing some melanophores obtained from pigmented regions. They found that the melanophores persisted and invaded the surrounding unpigmented dermis. They also found that autotransplants of scales and their adhering tissues produced no tissue antagonism while homotransplants of similar elements did.

More than twenty years ago Gordon (1931) found that macromelanophores had to be present in platyfish × swordtail hybrids before a melanoma would develop spontaneously. It was also known from histological studies by Reed & Gordon (1931) that the definitive melanoma of these fish contained every conceivable gradation of cells between the darker, larger melanophores



TEXT-FIG. 4. Schematic representation of the observations on the various fates of melanoma implants.

I. Soon after transplantation the implant is surrounded by a gray halo of free melanin particles.

II. Macrophages (or, melanophages) engulf and remove the melanin detritus.

IIa. Some implants, particularly the heterotransplants, degenerate within a short time, breaking up and forming masses of melanin debris.

IIb. Last stage of implant degeneration, in which most of the melanin debris has been eliminated by the action of melanophages.

III. Dendritic processes of pigment cells appear at the margin of the implant; most of the melanin detritus is removed.

IIIa. Some implants after reaching this stage of growth begin to degenerate.

IIIb. Most of the implants that fall into the category indicated by IIIa degenerate completely. One implant, however, persisted at this stage for several months and then regenerated to grow to the next stage, IV.

IV. The implant is surrounded by many dendritic processes and by whole pigment cells. This is a critical period in the growth of the implant because regression may still take place up to about six weeks after implantation.

V. The growing transplant, in which the white circle represents the size of the original implant. Around the periphery of the growth free melanophores and melanocytes may be identified. These are the primary cells of the melanoma. Histological study of such transplants reveals the presence of a melanosis, the precursor of melanoma development, rather than a definitive melanoma.

and the lighter, smaller, irregular (stellate) pigment-containing cells which are now known to be melanocytes. These authors suggested that the smaller cells are the precursors of the larger ones. There has been no direct evidence of this important cell relationship up to the time that observations were made on the ontogeny of pigmented cells in the transplanted melanomas, as indicated in this paper. These observations were confirmed almost simultaneously by the study of regeneration of melanomatous dorsal fins of platyfish × swordtail hybrids by Ermin & Gordon (1952, 1954). The evidence from both sources is still fragmentary, but the new techniques of transplantation and regeneration should in future work add to our knowledge of the relationships, that is, the histogenesis and ontogeny, of these vitally important cells of the melanoma.

Summary and Conclusions

1. Melanomas develop on the caudal peduncles and caudal fins of swordtail (*Xiphophorus montezumae* × *X. helleri*) hybrid fishes carrying the dominant macromelanophore gene for spotted caudal, *Sc*. Autotransplants of the melanomas have been successful. Homotransplants of the melanoma have also been successful in hosts having the dominant *Sc* gene. Transplants into other hosts either failed completely or were maintained only for short periods.

2. Transplantation of the tumor fragment into the corium under the scales and epidermis permits the continuous observation of individual cells in the growing implant, owing to the transparency of the thin tissues.

3. The histological characteristics of the implanted melanoma tissue (high vascularity, blood filled lacunae, lightly pigmented melanocytes) are not present in the well-established implant. After 24 months of activity, the original melanoma implant takes on the morphological features of a melanosis. Melanosis, a premelanomatous stage, is produced by the hypertrophic growth of macromelanophores.

4. Two types of immature pigment cells, or melanocytes, emerge from the implant. The smaller one (*small melanocyte*) is thin and has few and tenuous pseudopodial processes; the larger one (*large melanocyte*) is broad and has wide, blunt pseudopodia.

5. Some large melanocytes transform into macromelanophores.

References

Algire, G. H.

1943. Microscopic studies of the early growth of a transplantable melanoma of the mouse, using the transparent chamber technique. J. Nat. Cancer Inst., 4: 13-20.

Algire, G. H., & F. Y. Legallais

1948. Growth and vascularization of transplanted mouse melanomas. *In* The Biology of Melanomas. Spec. Publ. N. Y. Acad. Sci., 4: 159-170.

Ermin, Recai, & Myron Gordon

1952. Study of the regeneration process in fishes after amputations of dorsal fins with and without melanotic tumors. Cancer Research, 12(4): 260-261. (Abstract).

1954. Regeneration of melanomas in fishes. Zoologica, 39: (In press).

Fitzpatrick, T. B., & A. B. Lerner

1953. Terminology of pigment cells. Science, 117 (3049): 640.

Goodrich, H. B., & R. Nichols

1933. Scale transplantation in the goldfish *Carassius auratus*. I. Effects on chromatophores. II. Tissue reactions. Biol. Bull., 65: 253-265.

Gordon, Myron

1931. The hereditary basis of melanosis in hybrid fishes. Amer. Jour. Cancer, 15: 1495-1523.

1951. Genetics and correlated studies of normal and atypical pigment cell growth. Growth, Symposium, 10: 153-219.

1953. Preface. *In* Pigment Cell Growth. New York, Academic Press, Inc.

Gordon, Myron, & R. F. Nigrelli

1949. The genetics and pathology of the melanomas in the hybrid offspring of two species of swordtails, *Xiphophorus montezumae* and *Xiphophorus helleri*. Cancer Research, 9: 553. (Abstract).

Gordon, Myron, & G. M. Smith

1938. Progressive growth stages of a heritable melanotic neoplastic disease in fishes from the day of birth. Amer. Jour. Cancer, 34: 255-272.

Grand, C. G., & Gladys Cameron

1948. Tissue culture studies of pigmented melanomas: fish, mouse and human. *In* The Biology of Melanomas. Spec. Publ. N. Y. Acad. Sci., 4: 171-176.

Grand, C. G., Myron Gordon & Gladys Cameron

1941. Neoplasm studies. VIII. Cell types in tissue culture of fish melanotic tumors compared with mammalian melanomas. Cancer Research, 1: 660-666.

Krontovsky, A. A.

1916. On comparative and experimental pathology of tumors. Kiev. (In Russian). (Cited in E. A. Sheremetieva-Brunst & V. V. Brunst, *loc. cit.*)

Levine, Michael

1948. The cytology of the typical and the amelanotic melanoma. *In* The Biology of Melanomas. Spec. Publ. N. Y. Acad. Sci., 4: 177-215.

Marcus, T. R., & Myron Gordon

1953. Genetics of the *Sc* transplantable melanoma in fishes. Proc. Amer. Assoc. Cancer Res., 1(1): 36. (Abstract).

Reed, H. D., & Myron Gordon

1931. The morphology of melanotic overgrowths in hybrids of Mexican killifishes. Amer. Jour. Cancer, 15: 1524-1551.

Sheremetieva-Brunst, E. A., & V. V. Brunst

1948. Origin and transplantation of a melanotic tumor in the axolotl. *In* The Biology of Melanomas. Spec. Publ. N. Y. Acad. Sci., 4: 269-287.

EXPLANATION OF THE PLATES

Plate I

Fig. 1. *montezumae* × *helleri* swordtail hybrids of stock h50*Sc*, with large, spontaneously developed *Sc* melanomas on their caudal peduncles and tails. In these lateral views the full dimensions of the melanomas cannot be appreciated, but actually each tumor was approximately a quarter of an inch thick. Note, in the male above, the rough dorsal and ventral surfaces of the caudal peduncle, and in the female below, the thickened lower lobe of the tail. Note the absence of black markings in the anterior areas of the hybrids.

(Photographs of fishes all life size).

Fig. 2. Homotransplant in a female *montezumae* × *helleri* h42*Sc* hybrid, above, and an autotransplant in a male hybrid, below. In the female the implant, obtained from its sibling, was placed in the belly region. The black areas in the tail developed spontaneously in response to the action of the *Sc* gene in the host. In the male, the implant, obtained from its own caudal fin melanoma, was placed in the middle area of the left side of the body. The black marking in the dorsal fin and the melanoma of the tail developed spontaneously in response to the *Sc* gene.

Plate II

Fig. 1. Melanoma transplants in two male *montezumae* × *helleri* swordtail hybrids of strain h42*Sc*. In the male, above, the tumor tissue was removed from the melanoma in its tail and transplanted to its body just anterior and ventral to its dorsal fin. The hybrid was photographed 7 months after the implantation. In the male, below, the implant, obtained from its sibling, was placed just dorsal to the ventral fins and posterior to the pectoral fin. The other markings on the two fish are those spontaneously produced by macromelanophores in response to the *Sc* gene.

Fig. 2. An autotransplant and a heterotransplant of the spotted-dorsal melanoma originally developed spontaneously in response to the *Sd* gene in *helleri* × *maculatus* platyfish-swordtail hybrids. Above, an implant taken from the melanoma of the dorsal fin of a platyfish × swordtail hybrid was placed in the ventral region of the belly, just anterior to the ventral fins. The other melanotic areas developed spontaneously in response to the *Sd* gene. Below left, a melanoma taken from a platyfish × swordtail hybrid was placed by means of a trocar into the intermuscular region below the dorsal fin of an albino swordtail. To the right, the same albino swordtail showing a stage in the elimination (through the surface by means of macrophages) of most of the melanoma tissue. Both the heterotransplant and autotransplant were partially successful. (From a composite unpublished photograph of specimens studied by Gordon.)

Plate III

History of a melanoma transplant in a *montezumae* × *helleri* swordtail hybrid of strain h42*Sc*. Photographs made from the living fish shown in Plate I, Fig. 2. Magnification 48×.

Fig. 1. Transplant, 0.8 × 0.7 mm, photographed within 24 hours after insertion. Fine melanin particles surround the implant.

Fig. 2. Within 36 hours, melanoma cells have grown out from the transplant; this may be seen best at the ventral surface. Macrophages (forming 3 large black masses) with engulfed free pigment particles appear to the right, along the edge of the striated scale. Note the group of macrophages just to the right of the implant and follow it in Figs. 3 and 4.

Fig. 3. Within 48 hours, growth of melanoma cells may be seen at the ventral region where focus is better, but growth extends from the dorsal region of the transplant as well. The pigment-laden macrophages

may be seen lined up along the distal margin of a scale.

FIG. 4. Within 72 hours, the melanomatous mass has spread. The pseudopodial or dendritic processes of the pigment cells extend beyond the main mass; those at the ventral margin are filamentous, those dorsally are broader.

FIG. 5. After 8 days, many pigment cells have wandered out of the main mass. The free pigment has been removed by macrophages; note that only two of the three larger groups of macrophages remain and that they are considerably smaller.

FIG. 6. Melanoma transplant in a *montezumae* × *helleri* hybrid, h42*Sc*, photographed two weeks after implantation. To the left, a vigorous outgrowth of pigment cells consisting of melanocytes and macromelanophores may be seen. The entire implant is surrounded by many micromelanophores which are the normal pigment cells of the host.

PLATE IV

Figs. 1 to 4. Photomicrographs of pigment cells growing along the margin of living melanoma implants.

FIG. 1. Small melanocyte, characterized by its long, filamentous dendritic processes and relatively small cell body, seen to the left.

FIG. 2. Large melanocytes in a stage of transformation to melanophores. Note the concentrations of pigment granules (1) at the peripheries of the broad branches, and (2) at the center of the cell, particularly around the nucleus of the lowermost cell.

FIG. 3. Large melanocytes and a macromelanophore. Two melanocytes are shown along the upper part of the photomicrograph, another below and to the left. To the bottom right, partly overlain by the melanocyte, a large melanophore may be seen.

FIG. 4. Melanophores derived from melanocytes —the one to the left still retains some features of the melanocyte with its broad branching processes.

FIG. 5. Cross-section through epidermis and corial tissues between the scales of a transplanted melanoma fixed after 8 months of growth. The epidermis (top) is slightly hyperplastic and has a heavily pigmented layer between it and the scale. Between the two scales the corial tissues are completely replaced by macromelanophores.

FIG. 6. Cross-section through the skin and underlying muscles of a melanoma transplant fixed after 8 months of growth. In addition to details illustrated in Fig. 5, this photomicrograph shows the subcutaneous invasion of the muscles by pigmented cells derived from the transplanted melanoma. The direct path of pigment cell invasion may be seen to the right. This type of growth has a parallel in the manner in which spontaneous melanomas develop in platyfish-swordtail hybrids described and illustrated by Gordon & Smith (1938).

FIG. 7. Whole mount of the ventral surface of a scale with its adhering pigmented tissues. The scale was removed from a fish that had an actively growing melanoma transplant for one week. The dorsal edge represents the distal margin of the scale. Here a few macrophage groups with their contained melanin particles are passing out through the epidermis. Other pigment-containing macrophages and a few dentritic melanocytes may be seen in the relatively clear epidermal area. The lower two-thirds of the photomicrograph reveal the pigment cells that have grown out from the transplant into the dermal tissue. The dendritic cells are mainly macromelanophores and melanocytes, some of which have disintegrated. The roundish masses of pigment represent melanophages (macrophages).

PLATE V

FIG. 1. Section through an *Sc* melanoma in a swordtail hybrid. (Photomicrograph by Dr. Ross F. Nigrelli).

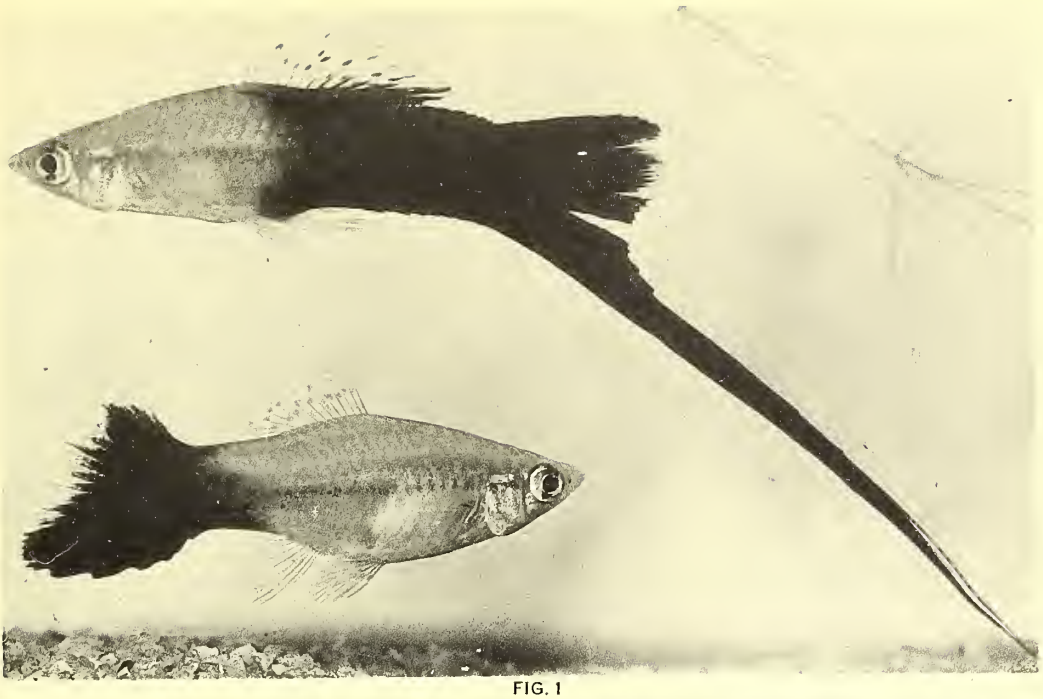


FIG. 1

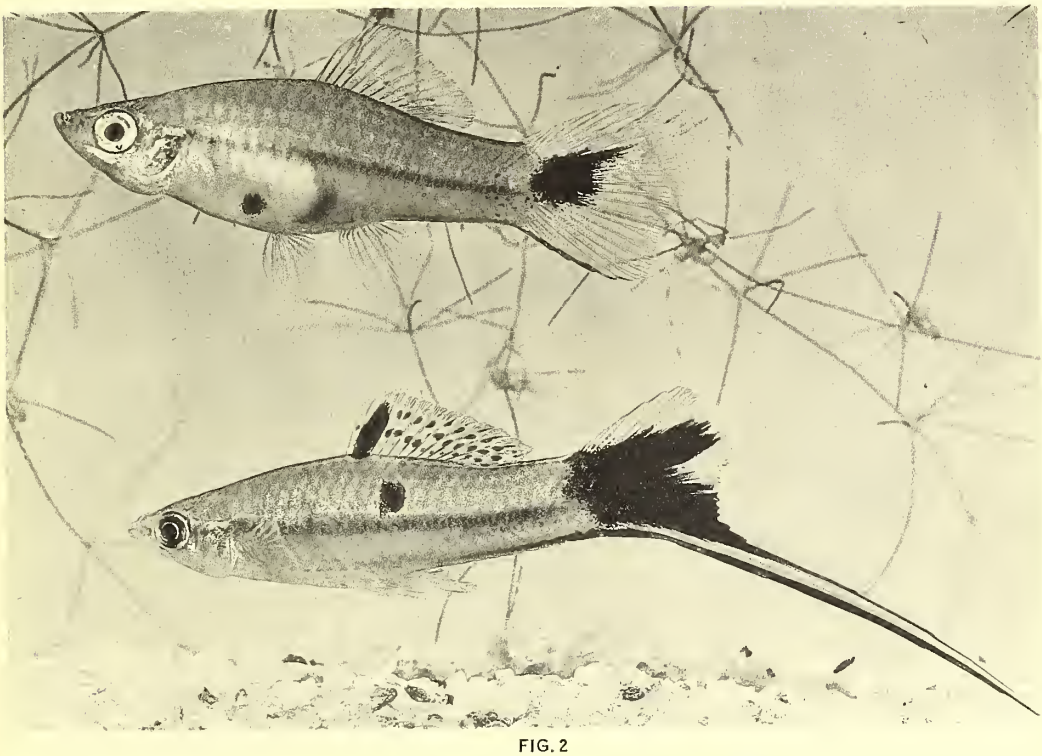


FIG. 2

TRANSPLANTATION OF THE *Sc* MELANOMA IN FISHES

FIG. 1

FIG. 2

TRANSPLANTATION OF THE *Sc* MELANOMA IN FISHES

FIG. 1

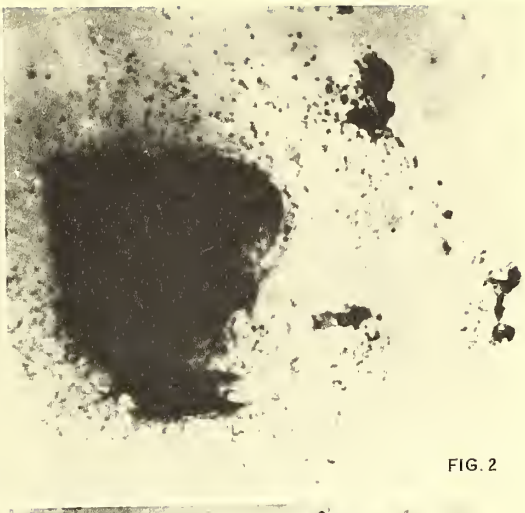


FIG. 2

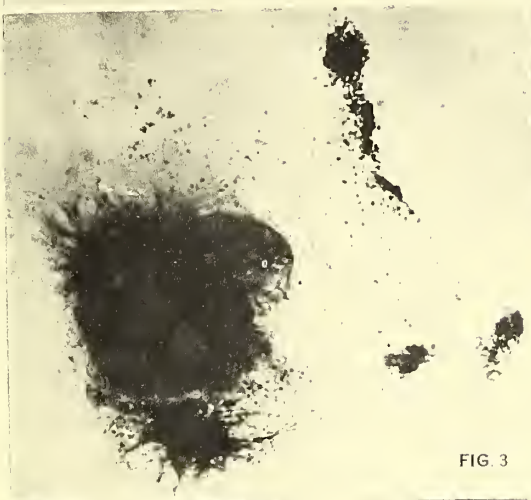


FIG. 3

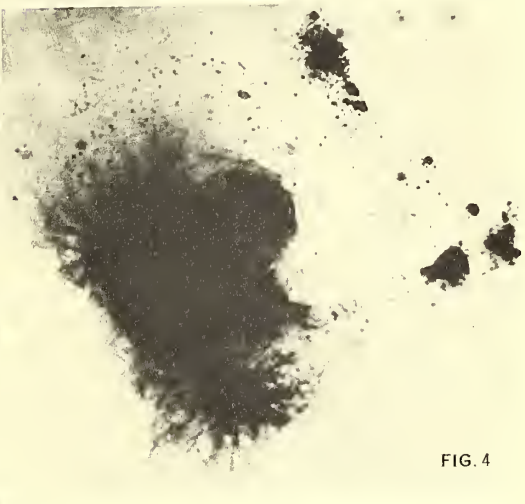


FIG. 4

FIG. 5

FIG. 6

TRANSPLANTATION OF THE *Sc* MELANOMA IN FISHES

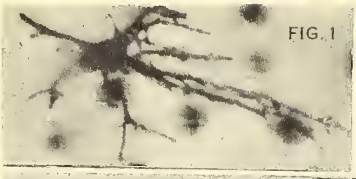


FIG. 1

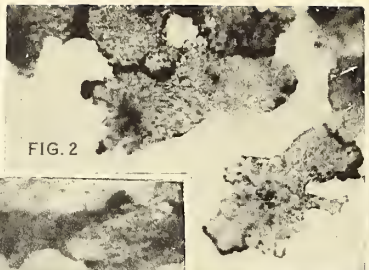


FIG. 2


FIG. 4

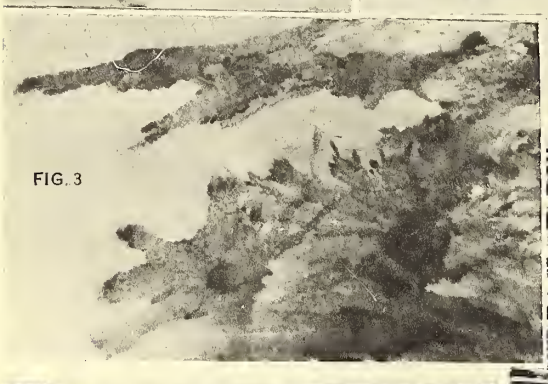


FIG. 3

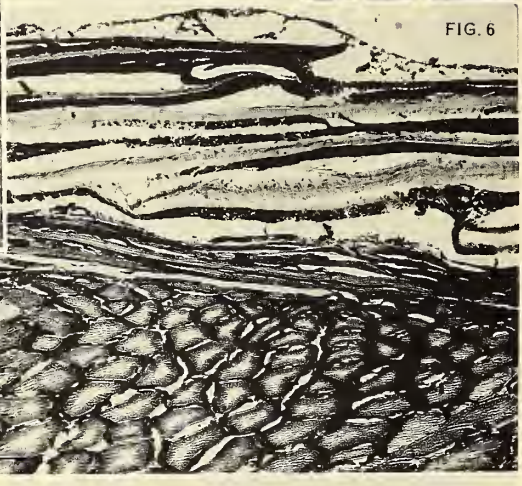


FIG. 6

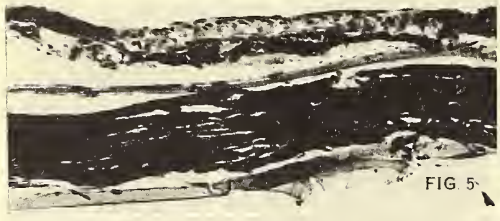


FIG. 5

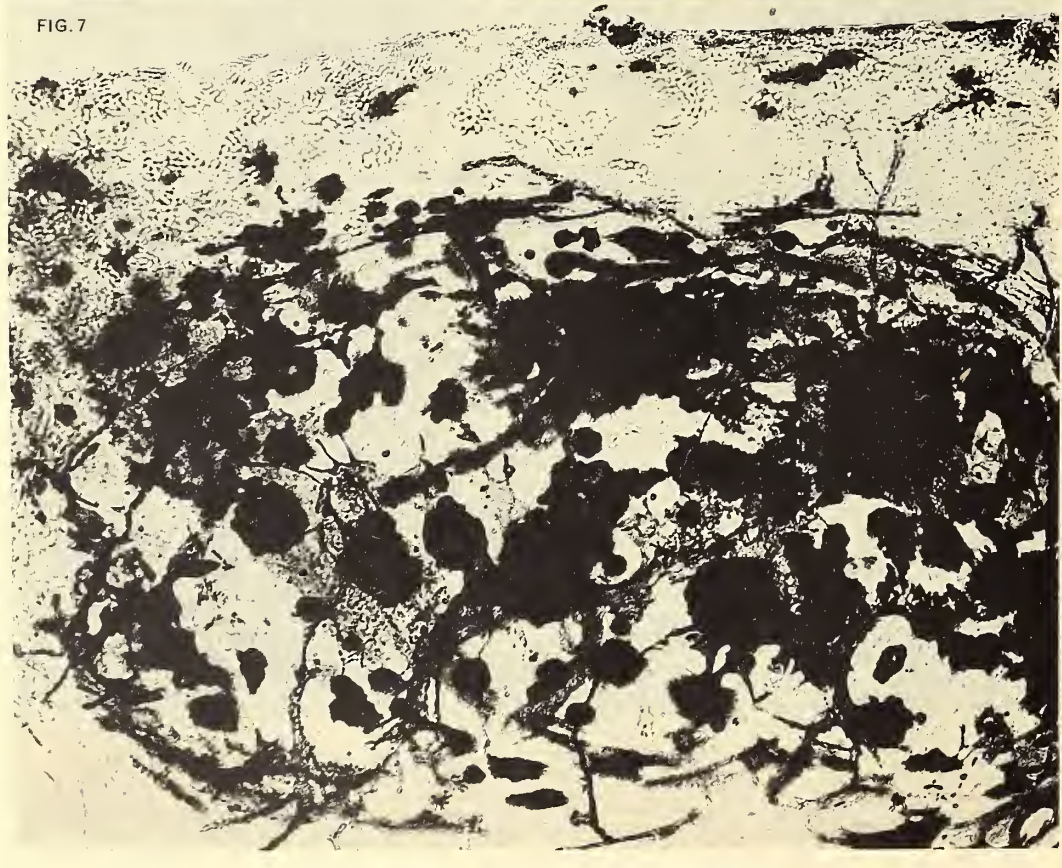


FIG. 7

TRANSPLANTATION OF THE *Sc* MELANOMA IN FISHES

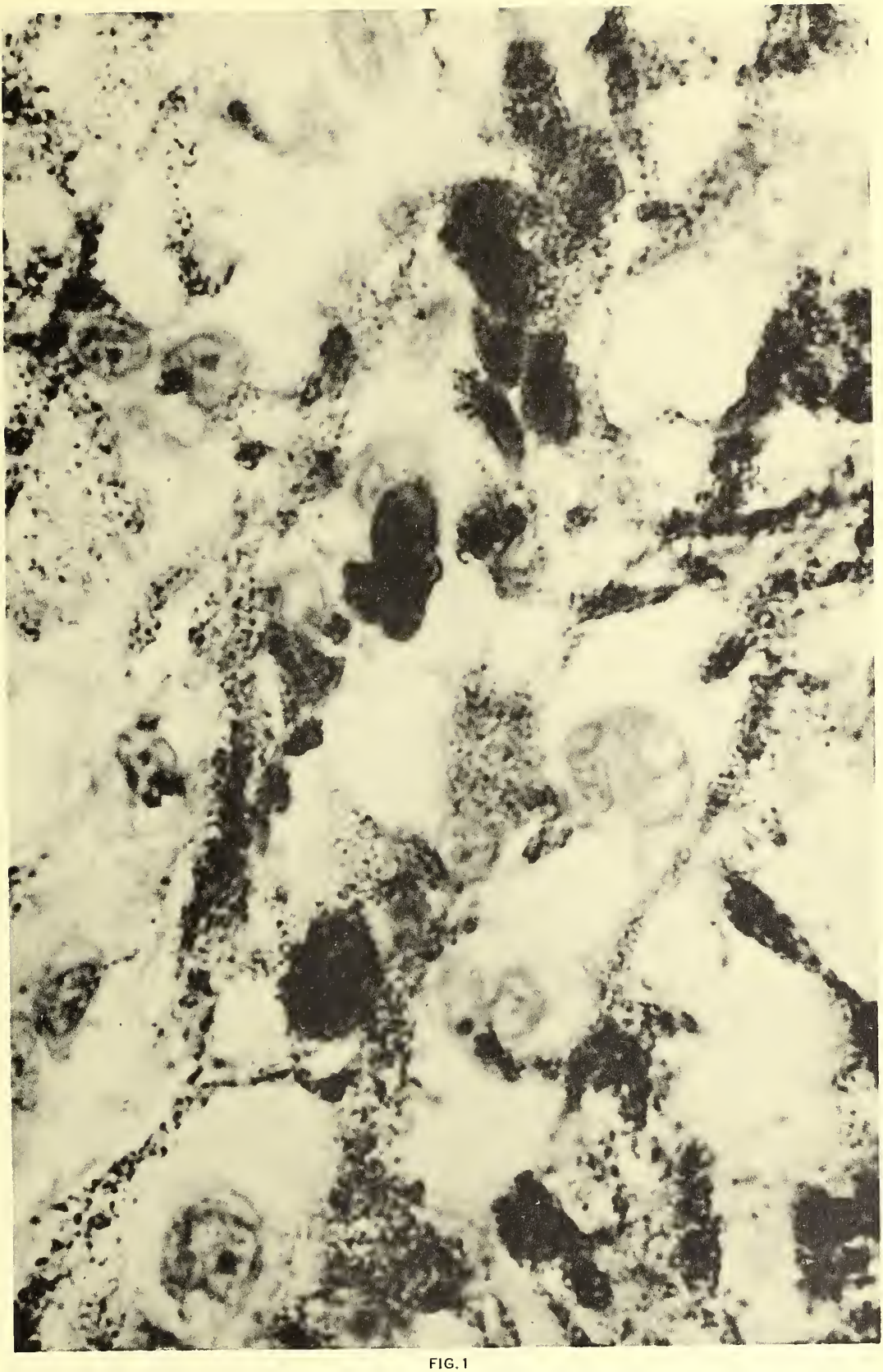


FIG. 1

TRANSPLANTATION OF THE *Sc* MELANOMA IN FISHES

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Contents

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The Effects of Forebrain Lesions on Mating Behavior in the Male Platyfish, *Xiphophorus maculatus*[1]

Robert P. Kamrin[2] & Lester R. Aronson

Department of Animal Behavior,
The American Museum of Natural History

(Text-figure 1)

In recent years numerous investigators have been concerned with the relation of the forebrain to sexual behavior in mammals (Beach, 1942, 1947), but comparable studies on lower vertebrates are limited. This is particularly true for teleosts. The live-bearing fishes are especially suited for a comparative study since reproductive behavior in these fishes consists of a series of patterns which are at least analagous to precopulatory and copulatory behavior in mammals. Among these fishes the poeciliid live-bearers such as the platyfish, swordtail and guppy are most useful, since their reproductive anatomy, physiology and behavior have been investigated to a greater extent than in any other teleost group.

Early investigators interested in forebrain function in fishes postulated that this region of the brain was primarily concerned with olfaction or with olfaction and its correlation with taste (Herrick, 1922). These concepts were based essentially on neuro-anatomical evidence from studies on the Chondrichthyes (cartilaginous fishes) and on certain of the more primitive Osteichthyes (bony fishes) where olfaction and taste play a predominant role in the sensory repertoire. Although numerous scattered reports, pointing to a more generalized facilitative function of the forebrain of fishes not immediately related to olfaction, have appeared during the last few decades, the early concept of olfaction is still adhered to quite widely.

Experimental studies on forebrain lesions and extirpations in fishes have been reviewed by Ten Cate (1935). Various investigators reported no changes in locomotion, equilibrium, vision, feeding or conditioned responses to optic stimuli. Electrical stimulation of the intact forebrain produced no motor responses. Olfaction was abolished, as would be expected. However, Vulpian (1866), Janzen (1932), Hosch (1936) and Berwein (1941) have reported a general loss of responsiveness which they variously termed reduction in "arbitrary movements," "initiative," "irritability," and the like. Kumakura (1928), Noble (1936) and Wiebalck (1937) observed that schooling, which is primarily a visually directed response, was impeded in forebrainless fishes of several species.

The first studies in fishes that were oriented directly toward explaining the function of the forebrain in sexual behavior were those of Noble (1936, 1937, 1939a, 1939b), who reported, in a series of abstracts, that lesions in the corpus striatum (cerebral hemispheres or olfactory lobes in our terminology) in several species of cichlid and poeciliid fishes resulted in a loss of synchronization between the male and female in spawning and parental care. Noble & Borne (1941) reported that unilateral forebrain ablation caused no discernible alteration in sexual behavior in the oviparous *Betta splendens* and *Hemichromis bimaculatus,* although bilateral extirpation of the telencephalon caused complete cessation of sexual activity. However, in the

[1] This study was supported in part by a grant from the Committee for Research in Problems of Sex, National Research Council. The authors wish to express their appreciation to the Department of Zoology of Cornell University for making available certain of its facilities during later phases of this work and during preparation of the manuscript. An expression of gratitude is extended to Dr. M. Singer of Cornell University and Dr. T. C. Schneirla of the American Museum of Natural History for helpful advice and criticism of the manuscript. The experimental fishes were obtained through the courtesy of the Genetics Laboratory of the New York Zoological Society.

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viviparous poeciliid *Xiphophorus helleri,* complete removal of the forebrain had no effect on mating behavior. Aronson (1948), investigating the specific acts comprising spawning in the West African mouthbreeding cichlid *Tilapia macrocephala,* reported that early courtship patterns were only slightly affected by hemidecerebration or total decerebration. Those patterns more immediately related to spawning were markedly reduced in frequency of occurrence, especially by the more drastic lesions.

Materials and Methods

The present study was performed on 27 sexually mature virgin male platyfish, *Xiphophorus* (*Platypoecilus*) *maculatus.* Throughout the experiment the fish were isolated in separate two-gallon aquaria, the rear and sides of which were painted an opaque blue to exclude external disturbances. The aquaria were situated in a greenhouse maintained at approximately 25° C.

Prior to operation, the males were deeply anesthetized in a 3 percent urethane solution and were then wrapped in a piece of cotton soaked in the anesthetic so that only the dorsal surface of the head was exposed. Under a dissecting microscope, an opening approximately 2 × 3 mm. was made in the roof of the skull between the eyes with a pair of iridectomy scissors, thus exposing the forebrain. Varying portions of the forebrain were then ablated with a low pressure aspirator. Bleeding was negligible. The fish were then placed in aquarium water to which 0.8% Louisiana rock salt had been added. Granulation tissue closed the wound within six days; epithelial coverage was completed in about nineteen days.

Observations of sexual behavior totaling ten pre-operative and ten post-operative tests were conducted, each period being ten minutes long. Clark, Aronson & Gordon (1954) found that if proper testing techniques are used, this interval is sufficient to obtain an adequate sample of sexual activity in this species. At the start of each test, a previously isolated virgin female (or a non-virgin that had been isolated for at least eight months) was placed in the male's aquarium. The frequency, time, and sequence of behavior events were recorded on a specially constructed twenty-pen Esterline-Angus graphic recorder (Clark, Aronson & Gordon, 1954). A lapse of eight days was allowed between the operation and the first of the post-operative tests that followed. During this period, all operates were grossly examined and appeared to have regained their health.

The behavioral patterns observed, as described by Clark, Aronson & Gordon (1954) and Schlosberg, Duncan & Daitch (1949), are summarized below. Each behavior is preceded by an abbreviation, which later accompanies the descriptions of the lesions.

Gonopodial swinging (Sw.)—a foreward movement of the male's gonopodium in conjunction with one pelvic fin; performed when the male is not swimming close to the female.

Thrusting (T.) (Tc = contact thrust; Tn = non-contact thrust)—a gonopodial swing directed toward the genital opening of the female. The gonopodium may or may not come in contact with the female's genital opening. During thrusting, spermatophores are not transferred to the female's genital tract.

Copulation (C.)—a prolonged contact thrust often resulting in the transfer of spermatophores to the female.

Pecking (P.)—a rapid series of biting movements at the gravel on the bottom of the aquarium.

Sidling (S.)—the male swims close to the female and tilts his body slightly so that his mid-ventral region is close to the genital area of the female.

Nipping (N.)—the male pursues the female and nips her body especially about the head and genital region; closely associated with aggressive behavior.

Retiring (R.)—usually after swinging or thrusting, a slow or rapid backward swimming away from the female until the male strikes some surface. The male then settles gradually to the bottom of the tank and remains quiescent before resuming activity.

Quivering (Q.)—a rapid up-and-down or side-to-side movement of the male's entire body, which is held in an S-shaped curve, the dorsal and caudal fins folded.

S-curving (Sc.)—an extreme tensing of the fish's body into a simple arc or S-shaped curve.

After each observed copulation, females were examined for sperm by an oviduct smear technique (Clark & Aronson, 1951; Clark, Aronson & Gordon, 1954). Inseminated females were not reused in tests.

Shortly after the last post-operative observation, the brains were removed, fixed in 10% formalin, embedded in paraffin and sectioned transversely at ten μ. Sections were stained with gallocyanin (Einarson, 1932). With a projection microscope, outline drawings were made of approximately every tenth section of each brain. By comparing these drawings with a similarly constructed normal series, the extent of

brain damage was estimated. The description of the operate lesions as well as an analysis of the normal neural configuration of the platyfish forebrain are presented here as an appendix to the paper.

The intact portions of the forebrains exhibited considerable plasticity after operation. This was particularly true after unilateral decerebration, where the remaining lobe was found to occupy a central position and the nuclear patterns were profoundly distorted. For this reason our attempts to estimate, by means of planimeter readings from the serial projections, the mass of forebrain ablated proved unreliable.

Results

The changes in behavior following forebrain deprivation are summarized in Table 1. The average score per test for the ten pre-operative tests for each item of behavior described above and for each fish are compared with the average post-operative scores. It may be noted that all of the sexual patterns except swinging decreased markedly in frequency of occurrence post-operatively. Copulation dropped most of all, appearing in low frequency in only three animals. On the other hand, thrusting behavior, which normally precedes copulation and is always a sign of a sexually aroused animal, dropped only about 33%, and swinging, which is also a sign of sexual excitability, did not decrease at all, even in some of the operates with the most extensive extirpations. Sidling, which usually precedes thrusting, decreased only slightly. Nipping, pecking and quivering, which are often referred to as courtship activities and are primarily synchronizing processes, declined only moderately. In contrast, S-curving and retiring, which are more closely associated with aggressive actions, were reduced most drastically and almost entirely disappeared from the males' repertoire. In five males (nos. 7, 9, 15, 17 and 44) there was a decided post-operative increase in swinging, sidling and thrusting, which are the best indicators of heightened sexual arousal.

There seemed to be a tendency for thrusting behavior to disappear completely or to be greatly reduced in frequency in those fishes in which the deprivation included all or most of the dorsal olfactory areas (nos. 11, 14, 17, 20, 41, 54, 57, 58). However, this distinction was not absolute and there was one outstanding exception, namely, male no. 17 whose forebrain had been completely ablated except for remnants of the preoptic nuclei. This operated male exhibited a high frequency of thrusting and other sexual patterns, and copulated once. Conversely, with the exception of male no. 17, whose thrusting scores increased after operation, an appreciable portion of the dorsal olfactory area remained intact. In those males with less extensive lesions, where a considerable portion of the dorsal olfactory area was uninjured, thrusting and other sexual activities decreased to a lesser extent. In four of the operated males (nos. 15, 16, 49, 51), one or both olfactory bulbs remained intact. As a group these four males did not differ in their sexual responses from those completely deprived of olfactory sensations.

There was no indication that the presence or absence of the preoptic area had any marked effect on the level and persistence of sexual behavior as suggested by Aronson & Noble (1945) in their work on the grass frog *Rana pipiens*.

During some observations, males nos. 5, 7, 19, 50 and 55 occasionally exhibited a peculiar parallel swimming movement which differed substantially from sidling. Thrusting never followed this aberrant behavior.

Discussion

Although critical experiments concerning sensory processes involved in sexual behavior in poeciliid fishes are not available, it is apparent to anyone working with these species that vision is of prime importance and that olfaction is of lesser importance. This is supported by our finding that several of the operated males exhibited a considerable amount of sexual activity, including copulation, in the complete absence or disruption of the olfactory apparatus.

The idea is still prevalent that the forebrain of fishes is primarily concerned with the organization of olfactory impulses, and for the same reason the prevailing terminology for many forebrain regions, nuclei and tracts includes the term "olfactory." One explanation for this apparent misconception is the over-generalization of the term "fishes." Actually, fishes are phylogenetically very old, and different groups of them have been following divergent evolutionary paths for a very long time. This is expressed in a great number of physiological and morphological differences, among them forebrain structure and function. It is clear that in many families of fishes, the olfactory function of the forebrain has become greatly limited or modified.

Vision is also poorly represented in the forebrain. Definitive fiber tracts from the tectal and diencephalic optic centers to the forebrain have not been demonstrated. Yet surgical invasion of the forebrain materially reduces what are thought to be essentially visually directed processes. On the other hand, the entire pattern of sexual behavior was elicited in a few instances

TABLE 1. AVERAGE FREQUENCY OF SEXUAL PATTERNS BEFORE AND AFTER FOREBRAIN OPERATIONS[a]

Male No.	Gonopodial Swinging		Thrusting		Copulation		Pecking		Sidling		Nipping		Quivering		S-curving		Retiring	
	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.	Pre.	Post.
3	7.7	12.5	4.7	2.6	0.0	0.0	4.4	3.2	3.4	2.6	5.9	0.1	0.5	0.0	0.2	0.0	0.3	0.1
5	11.6	10.7	70.4	37.1	0.6	0.0	14.8	6.1	17.5	19.2	2.4	0.1	0.6	0.1	0.1	0.0	0.9	0.1
7	2.5	10.5	18.3	22.1	0.3	0.0	0.9	5.0	6.6	13.2	0.3	0.1	0.2	0.3	0.2	0.0	0.3	0.0
9	2.5	8.6	0.0	16.4	0.0	0.0	0.1	0.7	0.2	7.8	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	5.9	0.0	19.4	0.0	0.3	0.0	0.7	0.0	2.6	0.0	0.0	0.0	0.4	0.0	0.1	0.0	0.2	0.0
12	7.5	3.6	16.3	0.1	0.6	0.0	2.3	1.8	6.9	4.3	0.3	0.1	0.1	0.2	0.6	0.0	0.8	0.0
13	15.6	11.2	5.5	21.0	0.2	0.0	8.7	1.2	4.5	10.2	0.0	0.1	0.2	0.0	0.4	0.0	0.2	0.0
14	13.5	0.8	11.4	0.0	0.1	0.0	4.0	0.3	2.8	0.0	0.9	0.0	0.0	0.0	0.2	0.0	0.0	0.0
15	9.5	11.6	16.9	19.6	0.2	0.0	3.1	2.6	6.0	8.7	1.3	0.1	0.0	0.1	1.4	0.1	0.0	0.8
16	9.4	8.7	5.3	3.7	0.4	0.0	2.3	1.3	5.0	2.6	1.4	0.1	0.4	0.0	1.0	0.0	0.3	0.1
17	4.6	8.1	16.7	95.9	0.9	0.1[b]	2.4	13.9	8.7	34.2	0.6	0.3	0.5	0.2	0.5	0.0	0.3	0.0
18	5.8	5.3	14.2	11.5	0.2	0.0	2.1	0.8	9.9	7.1	0.4	0.3	0.0	0.1	0.4	0.0	0.0	0.1
19	6.7	8.8	31.3	18.4	0.2	0.0	1.9	0.0	11.4	9.2	0.6	0.3	0.5	0.2	1.6	0.0	0.1	0.0
20	14.8	1.2	40.6	0.0	0.3	0.0	5.5	0.1	14.8	0.1	1.2	0.0	0.0	0.0	0.1	0.1	0.2	0.0
41	6.0	7.4	22.7	5.4	0.5	0.0	8.4	0.1	8.9	3.8	3.1	0.0	0.2	0.0	0.2	0.0	0.4	0.0
44	3.2	10.4	13.1	15.2	1.1	0.2[c]	3.6	4.4	4.5	3.8	0.6	1.1	1.0	0.2	0.1	0.1	0.3	0.2
45	9.5	7.8	40.2	20.6	1.2	0.0	5.4	0.7	19.6	5.1	0.0	0.2	0.7	0.0	0.4	0.1	0.8	0.0
49	6.2	7.2	9.5	2.4	0.0	0.1[c]	0.3	1.1	6.5	3.2	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0
50	4.2	5.9	33.8	0.0	0.0	0.0	0.2	0.5	11.4	3.7	0.0	0.0	1.5	0.0	0.4	0.0	1.1	0.0
51	5.6	10.0	135.0	7.0	1.5	0.0	2.1	4.7	16.7	12.9	2.6	6.1	1.3	0.8	1.1	0.1	1.0	0.0
53	19.7	12.0	131.6	23.5	1.1	0.0	0.5	0.0	41.3	13.5	0.5	0.0	0.7	0.0	0.3	0.0	0.6	0.0
54	5.7	10.5	32.7	0.0	2.1	0.0	0.1	0.4	13.9	0.1	0.4	0.0	0.6	0.0	0.5	0.0	1.1	0.0
55	8.2	7.4	38.4	1.9	3.1	0.0	3.0	0.2	15.8	1.5	0.1	0.2	1.5	0.0	0.6	0.1	2.3	0.0
57	12.8	19.6	23.9	0.0	0.8	0.0	1.7	0.1	10.6	0.6	0.4	0.1	0.6	0.0	0.4	0.0	0.8	0.0
58	7.0	11.5	49.1	3.7	1.1	0.0	0.0	0.4	16.5	3.8	0.2	0.1	0.0	0.0	0.6	0.0	0.9	0.0
59	11.9	10.0	25.4	0.5	0.7	0.0	0.0	0.4	10.6	1.3	0.8	2.6	0.6	0.0	0.5	0.0	0.9	0.1
61	9.7	13.9	10.3	0.0	0.0	0.0	0.0	0.3	5.1	0.3	0.6	0.0	0.0	0.0	0.1	0.0	0.1	0.1
Means	8.4	8.7	32.1	11.2	0.7	0.01	3.0	1.8	10.5	6.3	1.0	0.5	0.4	0.1	0.4	0.03	0.5	0.04
MEAN DIFF$\pm\sigma$	0.3 ± 1.1[d]		20.9 ± 7.2[e]		0.7 ± 0.2[f]		1.2 ± 0.9[d]		4.2 ± 1.8[e]		0.5 ± 0.8[e]		0.3 ± 0.1[f]		0.4 ± 0.08[f]		0.5 ± 0.1[f]	

[a] Based on 10 pre-operative and 10 post-operative observations, each 10 minutes long, for each male except male 19 which had only 9 pre-operative tests.

[b] Female not inseminated.

[c] Female inseminated.

[d] $P > .05$

[e] $P > .01 < .05$

[f] $P < .01$

in animals with total (or almost complete) absence of the forebrain.

These thoughts lead to the hypothesis that the forebrain does not function directly in the organization of sexual behavior patterns, but rather that it acts as a generalized sensitizer, or facilitator of centers and mechanisms lower in the brain. This is undoubtedly what certain of the earlier authors, mentioned in the introduction, described as loss of "initiative," etc. A facilitative action of the forebrain was also proposed in a study of brain function in relation to spawning in the mouthbreeding fish *Tilapia* (Aronson, 1948) and in the grass frog *Rana pipiens* (Aronson & Noble, 1945). Beach (1951) reached a similar conclusion following a study of brain injury and mating in male pigeons, and Beach (1942) and Lashley (1930) have extensive evidence for this type of action of the cerebral cortex of mammals. Herrick (1948), in a discussion of the evolution of the cerebral cortex, expressed the belief that this sensitizing action of the forebrain develops from parts of the original olfactory areas which lack localizing functions and to which ascending and descending pallial projection fibers were added during phylogenetic development. Thus it is evident that at least one fundamental component of mammalian cortical activity must have made its appearance very early in vertebrate history.

In an earlier study, Clark, Aronson & Gordon (1954) demonstrated that gonopodial swinging in male platyfish is directly correlated with thrusting and copulatory behavior. Males having low scores for swinging rarely thrust or copulated, and conversely those males with high scores for thrusting and copulation also exhibited a considerable amount of swinging. This behavior may therefore be used as an indicator of the degree of sexual arousal. These observations have a direct bearing on our finding in the present experiment that swinging scores were not adversely affected by forebrain deprivations. Thus we may conclude that for the most part the operated males were aroused to a degree equal to or better than before the operations, and the possibility that the proximity of the lesions to the pituitary gland may have adversely affected pituitary and gonadal function is thereby minimized.

Clark, Aronson & Gordon also found that gonopodial swinging is the only component of sexual behavior in platyfish which appears in completely isolated males. All the other sexual patterns are directly oriented toward the female (or another male). This is most likely a visual orientation, and it suggests once more that in these fishes the forebrain deprivations might be affecting primarily the visual processes associated with sexual behavior. The experiments of Aronson & Noble (1945) form an interesting parallel. In laboratory aquaria, male frogs implanted with one or more pituitary glands readily swim to, clasp and spawn with ovulated females. The first component, namely swimming to the female, is based on visual orientation. The remaining components are mediated by contact stimulation. Completely decerebrated frogs activated by pituitary implantation will not swim to the ovulated female even if she is close by. If the male should accidentally touch the female as he swims about the tank, however, he will rapidly turn and clasp the female, and then the rest of the spawning will proceed normally. Here, too, forebrain deprivation has its effect on the visual component of the sexual process. Finally we may recall the observation of Wiebalck (1937) who found that schooling, which is a visually directed response, was impeded in forebrainless fish.

Summary and Conclusions

Each of 27 mature male platyfish was paired with a mature female for ten tests, each of ten minutes duration. Quantitative records of various patterns of sexual behavior were made. Lesions of various dimensions were then made in the forebrains of all the males, after which the fish were given ten equivalent post-operative tests.

In general, all of the sexual acts except gonopodial swinging declined in frequency after operation, but a few males maintained a considerable level of sexual activity, even after extensive forebrain deprivation. There was no indication that any of the sexual patterns could be completely eliminated by forebrain removal.

It is concluded that the forebrain facilitates the activities of lower parts of the brain, particularly in relation to visually directed responses.

Bibliography

Aronson, L. R.

1948. Problems in the behavior and physiology of a species of African mouthbreeding fish. Trans. N. Y. Acad. Sci., Ser. II, 2: 33-42.

Aronson, L. R. & G. K. Noble

1945. The sexual behavior of Anura. 2. Neural mechanisms controlling mating in the male leopard frog, *Rana pipiens*. Bull. Amer. Mus. Nat. Hist., 86: 89-139.

Beach, F. A.

1942. Analysis of factors involved in the arousal maintenance and manifestation of sexual excitement in male animals. Psychosom. Med., 4: 173-198.

1947. A review of physiological and psychological studies of sexual behavior in mammals. Physiol. Rev., 27: 240-307.

1951. Effects of forebrain injury upon mating behavior in male pigeons. Behaviour, 4: 36-59.

Berwein, M.

1941. Beobachtungen und Versuche über das gesellige Leben von Elritzen. Z. vergl. Physiol., 28: 402-420.

Clark, E. & L. R. Aronson

1951. Sexual behavior in the guppy, *Lebistes reticulatus* (Peters). Zoologica, 36: 49-65.

Clark, E., L. R. Aronson & M. Gordon

1954. Mating behavior patterns in two sympatric species of xiphophorin fishes: their inheritance and significance in sexual isolation. Bull. Amer. Mus. Nat. Hist., 103, art. 2: 135-226.

Einarson, L.

1932. A method for progressive selective staining of nissl and nuclear substance in nerve cells. Amer. J. Path., 8: 295-308.

Herrick, C. J.

1922. Functional factors in the morphology of the forebrain of fishes. In, Libro en Honor de D. S. Ramón y Cajal I, Madrid: 143-202.

1948. The Brain of the Tiger Salamander, *Ambystoma tigrinum*. Univ. Chicago Press, Chicago.

Hosch, L.

1936. Untersuchungen über Grosshirnfunktionen der Elritze (*Phoxinus laevis*) und des Gruendlings (*Gobio fluviatilis*). Zool. Jb. Abt. 3, 57: 57-98.

Janzen, W.

1932. Untersuchungen über Grosshirnfunktionen des Goldfisches. Zool. Jb. Abt., 3, 52: 591-628.

Kappers, C. U. A., G. C. Huber & E. C. Crosby

1936. The Comparative Anatomy of the Nervous System of Vertebrates, Including Man. 2. The Macmillan Co., New York.

Kumakura, S.

1928. Versuche an Goldfischen, denen beide Hemisphären des Grosshirns exstirpiert worden waren. Nagoya J. Med. Sci., 3: 19-24.

Lashley, K. S.

1930. Basic neural mechanisms in behavior. Psychol. Rev., 37: 1-24.

Meader, R. G.

1939. The forebrain of bony fishes. Proc. Acad. Sci. Amst., 42: 657-670.

Noble, G. K.

1936. Function of the corpus striatum in the social behavior of fishes. Anat. Rec., 64: 34. Abstract.

1937. Effect of lesions of the corpus striatum on the brooding behavior of cichlid fishes. Anat. Rec., 70: 58. Abstract.

1939a. Neural basis of social behavior in vertebrates. Collect. Net, 14: 121-124.

1939b. The experimental animal from the naturalist's point of view. Amer. Nat., 73: 113-126.

Noble, G. K. & R. Borne

1941. The effect of forebrain lesions on the sexual and fighting behavior of *Betta splendens* and other fishes. Anat. Rec., 79: 49. Abstract.

Schlosberg, H., M. C. Duncan & B. H. Daitch

1949. Mating behavior of two live-bearing fish, *Xiphophorus helleri* and *Platypoecilus maculatus*. Physiol. Zoöl., 22: 148-161.

Sheldon, R. E.

1912. The olfactory tracts and centers in teleosts. J. Comp. Neurol., 22: 177-339.

Ten Cate, J.

1935. Physiologie des Zentralnervensystems der Fische. Ergebn. Biol., 11: 335-409.

Vulpian, A.

1866. Leçons sur la Physiologie générale et comparé du système nerveux. Germer Baillière, Paris.

Wiebalck, U.

1937. Untersuchungen zur Funktion des Vorderhirns bei Knockenfischen. Zool. Anz., 117: 325-329.

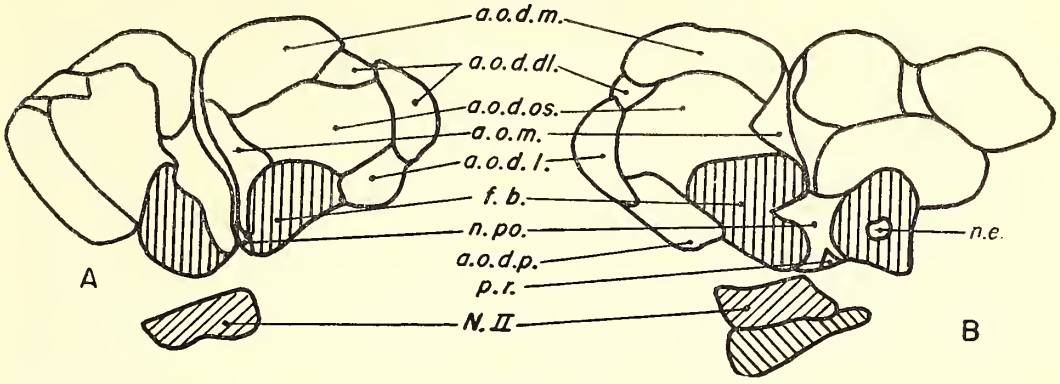
Appendix

A description of the forebrain of the platyfish is not available, nor has this region been studied in any related cyprinodont species. Therefore, in delimiting the extent of the lesions, the description by Kappers, Huber & Crosby (1936) of the nuclear configuration of the telencephalon of the sunfish was used. A few notable differences were observed:

1. The pars dorsolateralis of the dorsal olfactory area is much more extensive than in the sunfish and is subdivided into several discrete nuclei. Meader (1939) and Aronson (unpublished) have noted similar or more extensive enlargements and differentiations in a number of highly specialized teleosts.

2. The medial olfactory area is divided into several pre- and post-commissural nuclei.

3. The cells in the posterior lateral portion of the forebrain are distinctly separated from the pars lateralis of the dorsal olfactory area



TEXT-FIG. 1. Outlines of cross-sections through the forebrain of *Xiphophorus maculatus*, (A) anterior to the anterior commissure, and (B) posterior to the anterior commissure.

Abbreviations

a.o.d.dl. —area olfactoria dorsalis, pars dorsolateralis
a.o.d.l. —area olfactoria dorsalis, pars lateralis
a.o.d.m. —area olfactoria dorsalis, pars medialis
a.o.d.os. —area olfactoria dorsalis, pars olfacto-somatica
a.o.d.p. —area olfactoria dorsalis, pars posterior
a.o.m. —area olfactorius medialis
f.b. —medial and lateral forebrain bundles
N. II —optic nerve
n.e. —nucleus entopeduncularis
n.po. —nucleus preopticus
p.r. —preoptic recess

and are referred to here as the *pars posterior of the dorsal olfactory area*. This nucleus is probably homologous to the lobus pyriformis of Sheldon (1912) and has been variously named by other authors.

4. The pars magnocellularis of the nucleus preopticus cannot readily be distinguished from the parvocellular portion. This is atypical for teleosts.

The major nuclei of the platyfish forebrain that are referred to in the following descriptions of the lesions are shown in Text-figure 1. In the following descriptions of the lesions, the olfactory bulbs were either ablated, damaged beyond clear recognition, or were completely separated from the forebrain, except where specifically noted.

Male 3. (Sw, Tc, P, S, N, R)[3]. Dorsal olfactory areas ablated except for (1) pars posterior on right side, (2) a portion of pars posterior on left side and (3) portions of pars olfacto-somatica. Medial olfactory areas intact; preoptic nuclei intact.

Male 5. (Sw, Tn, P, S, N, Q, R). Dorsal olfactory areas ablated except for remnants of pars posterior and pars olfacto-somatica. Medial olfactory areas and preoptic nuclei intact. Slight invasion of anterior surface of right tectum.

Male 7. (Sw, Tn, P, S, N, Q). Dorsal olfactory areas ablated except for (1) remnant of pars posterior on right side and (2) small portions of pars lateralis, pars posterior and pars olfacto-somatica. Medial olfactory area invaded on right side; preoptic nuclei intact.

Male 9. (Sw, T, P, S). Left lobe of forebrain ablated except small part of medial olfactory areas. On right side dorsal olfactory area ablated except for (1) remnant of pars lateralis and (2) most of pars posterior. Right medial olfactory areas intact; preoptic nuclei intact.

Male 11. Forebrain completely ablated except for remnant of pars posterior of dorsal olfactory area and preoptic nuclei.

Male 12. (Sw, Tn-Tc, P, S, N, Q). Dorsal olfactory areas ablated except for small portions of pars olfacto-somatica and pars posterior. Medial olfactory areas almost intact; preoptic nuclei intact.

Male 13. (Sw, Tn, P, S). In right lobe, dorsal olfactory area extensively invaded at posterior pole, but pars medialis, pars lateralis and pars olfacto-somatica largely intact at the anterior end; in left lobe only remnants of dorsal olfactory area were found; medial olfactory area invaded dorsally on right lobe and completely destroyed on left lobe; preoptic nuclei intact.

Male 14. (Sw, P). Forebrain ablated except for small remnants of medial olfactory areas and pars posterior of dorsal olfactory areas; preoptic nuclei intact.

Male 15. (Sw, Tn-Tc, P, S, N, Q, R). On right side dorsal olfactory area destroyed except for remnants of pars olfacto-somatica. On left side dorsal olfactory area intact except for slight dorsal invasion; olfactory bulb intact. Dorsal portion of medial olfactory area invaded; preoptic nuclei intact.

Male 16. (Sw, Tn-Tc, P, S, N, R). Dorsal olfactory areas of both lobes mostly ablated; medial

[3] These abbreviations refer to the behavior patterns exhibited by each male in the post-operative tests.

olfactory areas almost completely intact; olfactory bulb on right side intact; preoptic nuclei intact.

Male 17. (Sw, Tn-Tc, C, P, S, N, Q). Forebrain entirely ablated except for small remnants of preoptic nuclei.

Male 18. (Sw, Tn, P, S, N, Q, R). On right side pars medialis of dorsal olfactory area ablated. Rest of dorsal olfactory area intact except for some invasion at the anterior pole; on left side dorsal olfactory area mostly ablated. Medial olfactory area of both lobes largely intact; preoptic nuclei intact.

Male 19. (Sw, Tn, S, N, Q). Dorsal olfactory areas destroyed except for the pars posterior and remnant of pars medialis. Medial olfactory areas ablated except for small remnants; preoptic nuclei intact.

Male 20. (Sw, P, S). Forebrain completely ablated except for (1) a small remnant of medial olfactory areas and (2) the preoptic nuclei.

Male 41. (Sw, Tn, P, S). Forebrain ablated except for (1) a small dorsal portion of the medial olfactory areas and (2) the preoptic nuclei.

Male 44. (Sw, Tn, C, P, S, N, Q, R, Sc). Dorsal olfactory areas ablated except for the pars olfacto-somatica and a portion of the pars posterior of the right lobe. Medial olfactory areas and the preoptic nuclei intact.

Male 45. (Sw, Tn, P, S, N, Sc). Forebrain completely ablated except for (1) remnants of the pars posterior of the dorsal olfactory areas, (2) portions of the medial olfactory areas and (3) the preoptic nuclei.

Male 49. (Sw, Tn, C, P, S, N, Q). Right lobe ablated except for (1) a portion of medial olfactory areas and (2) the preoptic nuclei. Left lobe intact except for slight invasion of pars lateralis of dorsal olfactory area. Left olfactory bulb intact.

Male 50. (Sw, P, S). Forebrain ablated except for (1) parts of the medial olfactory areas, (2) remnants of the pars olfacto-somatica and pars posterior of the right dorsal olfactory area and (3) the preoptic nuclei.

Male 51. (Sw, Tn, P, S, N, Q, Sc). Left lobe ablated except for (1) a remnant of pars lateralis of dorsal olfactory area, (2) most of medial olfactory area and (3) the preoptic nuclei. Right lobe intact except for slight lesion of pars medialis of dorsal olfactory area. Right olfactory bulb intact.

Male 53. (Sw, Tn, S, Sc). Dorsal olfactory areas ablated except for the pars olfacto-somatica, the pars posterior and a portion of the pars lateralis of the dorsal olfactory area of the right side. Most of the medial olfactory areas and the preoptic nuclei intact.

Male 54. (Sw, P, S). Forebrain completely ablated except for caudal remnant of the preoptic nuclei; habenular nuclei and anterior dorsal edge of diencephalon destroyed; slight lesion in anterior pole of left tectum.

Male 55. (Sw, Tc, P, S, N, Sc). On right side dorsal olfactory area ablated except for remnant of pars posterior. On left side portions of the pars medialis, pars lateralis, pars posterior and pars olfacto-somatica remain. Medial olfactory areas mostly intact; preoptic nuclei intact.

Male 57. (Sw, P, S, N). Forebrain completely ablated except for caudal ends of preoptic nuclei.

Male 58. (Sw, Tn, P, S, N). Same as 57.

Male 59. (Sw, Tc, P, S, N, R). On right side dorsal olfactory area ablated except for olfacto-somatica area and pars posterior which are largely intact. On left side dorsal olfactory area completely missing. Medial olfactory areas mostly intact; preoptic nuclei intact.

Male 61. (Sw, P, S, R). On right side dorsal olfactory area ablated except for remnants of pars lateralis, pars posterior and pars olfacto-somatica. On left side dorsal olfactory area ablated except for remnant of pars posterior. Medial olfactory areas mostly intact; preoptic nuclei intact.

12

Observations on the Spawning Behavior and the Early Larval Development of the Sargassum Fish, *Histrio histrio* (Linnaeus)

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(Plates I-III)

Introduction

LITTLE is known of the spawning habits and larval development of pediculate fishes. The larval development of *Lophius piscatorius* Linnaeus has been studied more than that of the others (Proctor, 1928), but there is no report of either spawning behavior or larval development of any of the antennariids. It is a fairly common occurrence, however, for solitary sargassum fish, *Histrio histrio* (Linnaeus), in small aquaria, to produce rafts of unfertilized eggs very similar to those of *Lophius*. There has been no report of females having been paired, nor has any record been made of the collection of such rafts of eggs from the natural habitat in open water with a description of subsequent larval development, although the work of Proctor on *Lophius* employed such a naturally-occurring raft.

At one time there was confusion as to the identity of eggs thought to belong to *Histrio*, which were found in balls of sargassum weed, but Gudger (1937), in his historical survey, shows these to belong to flying fish and reviews the relatively numerous notes of egg rafts produced by *Histrio histrio* (*Pterophryne gibba*) in captivity. In the present paper, the taxonomic usage of Barbour (1942) has been followed.

It is well known that the sargassum fish is a voracious feeder and does not hesitate at cannibalism. Even with an adequate food supply present in a tank containing two or more sargassum fish, it is only a matter of a few days before only one will remain, the others having been eaten. Because of this cannibalism, individuals of this species are generally kept alone in aquaria. Thus, although there is no difficulty in obtaining eggs under laboratory conditions, there is no record of pairing an ovulating female with a male.

In addition to the well-known egg rafts of the sargassum fish, Hornell (1921) reports that the closely related Indian species, *Antennarius hispidus* (Bloch & Schneider), occasionally spawned in the tanks at the Madras Aquarium, producing rafts of eggs very similar in his description to the egg mass of *Histrio histrio*. Here, too, however, the production of these egg rafts was by lone females and there is no record of fertilized eggs.

The studies here reported on both *Histrio histrio* (Linnaeus) and *Antennarius multiocellatus* (Cuvier & Valenciennes) were carried out at The Lerner Marine Laboratory, Bimini, Bahamas. My thanks are due Mr. Marshall Bishop, who succeeded in successfully pairing the fish and who made many helpful suggestions aiding in the raising of the young. Miss Priscilla Rasquin made careful observations of a second pair, independent of my records, and kindly submitted her data to me, greatly adding to an understanding of the typical behavior of these fish. I also wish to acknowledge the aid of Dr. C. M. Breder, Jr., in reading and criticizing the manuscript.

Laboratory Studies

The Egg Raft

In his records of egg production by *Pterophryne* (*Histrio*), Gill (1908) defines the spawning period as extending from July through October. However, spawning activity does not seem to be confined to any one time of year. Breder (1949) reports an egg-laying schedule of one female which extended from March to the middle of May. The spawnings here re-

ported were observed from early January to the beginning of March. From these scattered records, no clearly defined spawning season is yet apparent.

The egg mass of *Histrio histrio* (Plate I, Figure 1) is transparent and glassy in appearance. When freshly spawned, it is very firm, having a distinctive form, and is about 3½ inches long without being spread out. This egg mass has been variously described as a band, raft or sheet of eggs. Its most distinctive feature when freshly spawned is the form of the two scrolled ends. From observation of the rafts spawned in captivity, it appears that the normal position of the raft is that of floating with the two ends rolling up and toward the middle of the raft, the left-hand scroll spiralling clockwise, the right-hand scroll spiralling counter-clockwise. There is no marked straight part of the raft between the two rolled ends; rather the band of eggs forms a broad smooth curve from one scrolled end to the other. The egg raft cannot be straightened out, as in unrolling a flat piece of paper, for the two edges curve slightly upwards at right angles to the length of the raft.

When samples of the egg raft are examined microscopically, it is apparent that the eggs lie in several layers throughout the mass and that each egg is separated from the others by a well-defined membrane, which appears to correspond closely to the true chorion found in perch eggs as discussed by Nelson (1953) and as described for *Perca americana* (Schranck) by Ryder (1887). As in the perch, the *Histrio* egg is free to rotate within the chorion, but there are no spaces between the membranes of adjacent eggs. The eggs in their membranes are tightly packed in the raft, the surfaces of the chorion pressed into irregular planes about the egg. In the four-day period of larval development before hatching, the firm framework formed throughout the egg mass by these chorionic membranes gradually becomes less sharply defined. As the membranes deteriorate, leaving only an incomplete mesh, the whole egg raft softens and swells to about three times its original size (Plate I, Figure 2). Although the raft is extremely buoyant when freshly spawned, it begins to sink slowly. Even at the time of hatching, however, the ends of the raft still maintain a modified scrolled form.

During the time at which the sargassum fish were exhibiting such great spawning activity, a small female *Antennarius multiocellatus* was kept alone in an aquarium. Over a period of several days this female swelled to enormous size (Plate I, Figure 3). She finally released an egg raft (Plate I, Figure 4) very similar to those of *Histrio histrio*. Following the production of this egg raft, the *Antennarius* was once more reduced to very small size (Plate I, Figure 5).

The *Antennarius* egg raft showed scrolls at either end very similar to the distinctive form of the *Histrio* raft. In contrast to the *Histrio* egg mass, however, the middle of this raft appeared much longer and was relatively straight, not curving into the scrolls except at its extremities. Microscopically the structure of the raft is very similar to that of the *Histrio* raft, each egg being surrounded by a well defined membrane and the eggs in their membranes being tightly packed throughout the raft.

In his description of the structure of the *Lophius* egg mass, Proctor (1928) reports that "The embryos lie in a single layer in the mucus of the veil in capsule-like spaces containing from one to three or four eggs." These eggs were collected and first observed when they were beginning to form an embryonic shield. In the development of the *Histrio* egg, the breakdown of the chorionic membrane was, in one case, first noted just previous to the formation of the embryonic shield. It seems very possible that in the freshly spawned egg mass of *Lophius* there is a chorionic membrane encapsulating each egg, as was found to be true of the *Histrio,* and that by the time Proctor made his first observations of the *Lophius* veil, the firm membranes had begun to soften and become irregular.

Courting and Spawning Behavior

In all, five of the seven spawnings reported were closely watched from the first signs of courting behavior to the actual spawning. The spawnings watched all occurred in the late afternoon and early evening. The typical courting behavior became apparent in the early morning and continued through the day until spawning.

Egg Laying Cycle: First Female.—Two sargassum fish had been kept in laboratory aquaria for several months following their collection from floating weed. According to standard aquarium procedure in handling *Histrio,* as previously noted, each fish was in an individual tank. After several months in the laboratory, one of these fish produced an egg raft. On the same day, this known female was put into the tank in which the other *Histrio* had been kept. Up to this time, this second fish had produced no egg rafts in the laboratory aquarium, but there was no striking difference in the appearance of the two fish that would lead to identification of the male of the species. When the two fish were put together, neither one exhibited aggressive behavior towards the other. The second fish hovered close to the female constantly,

but made no attempt to attack or bite her. After three days together, the female produced another egg raft which was fertilized by the second fish. Following this, the pair spawned regularly every three days for more than two weeks. In all cases except the first egg raft production noted in the schedule below, the male was present and fertilized the eggs.

January 3rd, between 8:00 and 9:00 a.m.
January 6th, 4:35 p.m.
January 9th, 7:20 p.m.
January 12th, 8:35 p.m.
January 15th, 7:55 p.m.
January 18th, 7:15 p.m.
January 22nd, between 5:30 p.m. and midnight.

The egg masses were produced in very rapid succession and with a considerable amount of regularity, as may be seen by the above schedule. In a period of twenty days, seven egg masses were produced. Excepting the last spawning reported in the above schedule, there was a spawning every three days with a variation of only a few hours. Breder (1949) reported an egg-laying schedule for *Histrio* which included eight egg masses, the intervals between egg mass production varying from three to twelve days. This fish was a lone female and the eggs were not fertilized. The stimulation of the presence of the male may account for the rapid and regular production of eggs, three days, *i.e.* seventy-two hours, representing the minimal time for recovery from egg production and spawning to the maturation of the succeeding egg mass. The fish were not separated after each spawning, but left together in the same tank until after the fifth spawning, January 18th.

1st Spawning, January 6th.—The night previous to spawning, both fish of the pair exhibited normal tank behavior, hanging in the sargassum weed floating in the tank, drifting about and occasionally stalking and feeding on the live atherinids which were kept in the aquarium for that purpose. Although they did not show the actual courting behavior later found to be typical, the male maintained an attentive attitude towards the female, the two fish keeping close together most of the time. By early morning of the day of spawning, both fish showed marked changes in behavior. The female took up a position in a corner of the tank and did not move out from it at all throughout the day until the actual spawning. There was a shell to which a streamer of sargassum weed had been attached in the corner which the female adopted, and she spent the entire day holding onto the shell and weed with her pectorals, midway between the bottom of the tank and the surface of the water. In the early morning, about eight hours before egg laying, the female showed some abdominal distention. This enlargement increased steadily throughout the day until spawning. In the early morning, the female maintained an almost horizontal position, but as the abdominal enlargement progressed, she gradually changed to an almost vertical position, snout tipped downwards (Plate II, Figure 1). Several hours before spawning the female exhibited very rapid and heavy respiration which lasted until after spawning. The female maintained a light yellowish tan color which closely matched the weed and is the typical color exhibited by sargassum fish kept in aquaria. Throughout the day, she kept her dorsal and caudal fins and first dorsal spines stiffly erected.

The male maintained a position close to the female, only once or twice during the day moving out from the corner. He was a much darker color than the female, almost a chocolate brown. The male kept both dorsal spines and dorsal fin tightly retracted to the body. He was much more restless than the female, circling her and nudging her with his snout, but never leaving her immediate vicinity. In the last few hours before spawning, the male became increasingly more active, moving constantly about the female, and occasionally nudging her with his snout and pushing at her with his pectoral fins.

Immediately previous to the spawning, the female moved out from her corner into the open part of the tank with the male following close behind. The female then began a march, head tipped down, appearing to walk across the sand bottom of the tank on her ventral fins. The male followed close behind, with his snout in immediate contact with the female's vent. In this manner the two fish marched back and forth the length of the tank about four times, the male maintaining his position relative to the female even when making sharp turns (Plate I, Figure 5). Then together they turned upwards, dashed to the surface, and the egg mass appeared to burst from the female. The time interval between the fish leaving the corner of the tank and the completion of the actual spawning was not more than two or three minutes. The spawning itself did not take more than a few seconds. Immediately after egg laying, the female appeared in an extremely exhausted condition. She was observed in disorganized spiralling about the tank, but during the following night both fish fed.

2nd Spawning, January 9th.—Previous to the second egg laying, the courting activity was similar, with little variation. The female occupied the same corner in the tank as before, maintained the typical light color, and kept dorsal and caudal fins well spread and dorsal spines

erected. Before spawning, the female did not exhibit the extremely rapid and heavy respiration shown previous to the first spawning, although respiration did increase to some extent. The male showed the distinctive chocolate brown coloration all through the courting period. He kept both dorsal spines depressed, but occasionally throughout the courting period he erected and depressed his dorsal fin. The male appeared more restless than in the first spawning, making short excursions across the tank and back, but his activity was always closely oriented to the female. The male exhibited increased activity as before just previous to spawning, pushing at the female with snout and pectorals.

This spawning followed much the same pattern as the previous one. The two fish came out of the corner together, the female appeared to walk across the sand on her ventral fins, snout down, with the male swimming in a horizontal position behind her with his snout closely applied to her vent. The fish made about five trips back and forth across the tank. During this march, the male kept his dorsal fin well spread. The female exhibited sporadic tremors. The male appeared to push the female ahead of him to within a few inches of the surface, then turned away and swam past her, breaking the surface as he passed above the female. This spawning was photographed at 64 frames/second. The motion pictures show the egg raft stretched from the female up to the surface of the water (Plate II, Figure 7). The count of the motion picture frames shows the contact of the male and female lasted only 0.4 seconds, from the time at which the two fish started from the bottom of the tank, dashed to the surface and the male passed the female and headed back to the bottom. The film shows that the female remained at the surface for an additional 2.5 seconds with the egg mass extruded but not completely detached from the vent, before shaking free of the egg raft and starting to return to the bottom of the tank. Immediately following this spawning, the male became much lighter in color, closely matching the female. The female did not exhibit the total exhaustion shown in the previous spawning, but settled to the bottom of the tank quietly, and within ten minutes after the spawning both male and female were stalking and feeding on the atherinids in the tank.

3rd Spawning, January 12th.—About twenty-four hours previous to this spawning, both fish fed extensively, together consuming at least ten atherinids. By early morning of the day of spawning, the female had once more settled down, occupying the same corner of the tank as before. The male was even more restless than previously, making frequent trips back and forth across the tank, although these trips seemed oriented in relation to the female. Although the male made no attempt to feed on the atherinids present in the tank throughout the courting period, he exhibited aggressive activity towards foreign objects placed in the tank. If a hand was dipped into the water, he would dash at it vigorously.

About ten minutes previous to spawning, the male became very rough and aggressive towards the female, butting her forcibly with his snout and shoving her with his pectorals. The female appeared to make some attempt to avoid the male by pushing him away with her pectorals. Just before spawning, the two fish moved out of the corner of the tank together. The female did not settle to the bottom and march on her ventral fins as before, but swam slowly a few inches above the bottom, snout down. The male took up his typical position, swimming horizontally below and behind the female with his snout applied to her vent (Plate II, Figure 5). As the two fish swam slowly from one end of the tank to the other two or three times, the female exhibited the sporadic trembling which had been seen before. The egg raft appeared as the male pushed the female towards the surface (Plate II, Figure 6), but the female seemed to have difficulty in eliminating it, twisting and shaking herself at the surface. The male dashed quickly past the female, turning sharply away from her and swimming up, breaking the surface of the water. The actual contact of the two fish during this spawning was considerably longer than in the previously observed spawnings. The interval between the fish starting for the surface and the male heading back to the bottom was 2.8 seconds, this time being determined by a count of the motion picture frames covering the spawning. The female remained at the surface with the egg raft still streaming from the vent for an additional 6.6 seconds (Plate II, Figure 8). After the female had freed herself from the egg raft she appeared totally exhausted, having convulsions and spiralling about the tank for several minutes. Within half an hour, however, both male and female were quietly resting on the bottom (Plate II, Figure 9).

4th Spawning, January 15th.—The day previous to this spawning, the tank had been cleaned and the shell with its attached weed in one corner of the tank had been shifted slightly. In all previous spawnings, during the courting period the fish had hovered behind this shell and weed, which was the only protection available on the bottom of the tank.

In the morning before this fourth spawning, however, the female was in a position at the opposite end of the tank, braced with the aid of her pectoral fins between the standpipe and the end glass of the aquarium. However, to make photography possible the fish were disturbed and, with the aid of a glass plate, were crowded out of this end of the aquarium. They then immediately moved back into the corner with the shell and weed and hovered there, following the typical courting procedure. A few hours before spawning the male became excessively aggressive towards the female, shoving her with his snout and pectorals until finally, about a half hour before spawning, he appeared to force her from the courting corner out into the open part of the tank. As the male followed the female out of the corner, his color lightened from the typical chocolate brown of the courting period to a yellowish tan closely matching the color of the female. The male spread his dorsal and caudal fins extensively and periodically raised his dorsal spines. For the next thirty minutes the male persistently tried to get his snout near the female's vent in the typical position taken immediately before spawning, but the female pushed him away with her pectorals, at the same time slowly turning so that she faced him, then backed away slowly (Plate II, Figure 3). After thirty minutes the spawning occurred. Although not actually witnessed, the fish were heard splashing the surface of the water as they went to the top of the tank. The female showed no exhaustion immediately following the spawning and both fish fed within half an hour.

5th Spawning, January 18th. — The fish adopted the original corner of the tank behind the shell for the courting period. They exhibited the typical courting behavior, although the male appeared even more restless than before, continually making excursions across the tank and back. About an hour and a half before spawning the shell was moved out of the corner of the tank. The fish followed it out a way, then immediately returned to the corner. Twenty-five minutes before spawning, the male drove the female from the corner by forcibly butting and shoving her with his snout and pectorals. As the fish came out of the corner, the male lightened to a color matching the female. For the following twenty-five minutes the male alternately chased the female rapidly from one end of the tank to the other and sat quietly while the female rested. Several times during the chase, the male caught up with the female, took her entire caudal fin in his mouth and gave her a very violent shaking (Plate II, Figure 4). At one time, he succeeded in catching the female by the anal fin and again shook her violently. No apparent damage was done to the female, but after a rapid chase and shaking the female was exhausted and would settle to the bottom. The male would leave the female for a few minutes and make several excursions across the tank, then return and start to nudge the female with his snout again. The female would fend him off with her pectorals, turning to face the male, until finally the male would force the female to swim ahead of him and once more begin to chase her. During this chasing the male became even lighter in color, almost pinkish.

After a short rest period, the female came up off the bottom slowly, the male came up behind her and put his snout close to her vent in the typical pre-spawning position. The fish swam slowly in this manner for not more than a minute, then started for the surface. As they started up, a bit of the jelly mass of the egg raft could be seen protruding from the female's vent. As the female reached the surface, the male turned away and swam up past her. The female remained at the surface for about a minute, shaking and twisting in an attempt to free herself from the egg raft. In this interval, instead of heading back to the bottom, the male made a short turn, coming up once more below and behind the female. From this position the male made another dash past the female as if fertilizing for the second time. The male then swam to the bottom, and the female settled down slowly with the egg raft still attached and apparently only about two-thirds eliminated. The female remained quiet several inches off the bottom with the egg raft streaming from her vent behind and above her. Her respiration was very heavy and rapid. The male came over slowly, approaching the female from below and behind. As his snout neared the female's vent, the male opened his mouth wide so that as he moved forward the egg raft lay across his jaws. He made no attempt to bite the egg raft but swam with it streaming across his jaws. As the egg raft came into contact with his jaws, the male turned away from the female and swam up past her, the egg raft running through his mouth. As the male swung away from the female, the egg raft was brought into position lengthwise under the body of the male. The male did not continue to the surface of the water but swam away from the female as the end of the egg raft passed through his mouth. The relative positions of the two fish and the path of the

male as he passed the female appeared identical to those observed in actual spawnings. Following this, the female appeared completely exhausted, and she slowly drifted over to a corner of the tank and settled down quietly with the egg mass streaming behind her. The male appeared to lose all interest in the female for several minutes. He changed to the typical yellowish color and started making frantic dashes across the tank. Shortly afterwards, the male quieted and approached the female again. He passed her, as described above, coming up behind the female and carrying the egg raft upwards across his jaws. Following this, the male once more became very active, dashing back and forth across the tank. The female moved slowly across the bottom of the tank, finally settling down in one corner. The male returned to the vicinity of the female and his behavior closely resembled that of the courting period. He turned a chocolate brown color, partially depressed his dorsal fin and moved slowly about the female. The female remained quite still, with her tail tipped up slightly, and the egg raft streaming above her. About three hours after the raft first appeared, the female suddenly shifted to a position on her side on the bottom. She remained in this position for almost fifteen minutes, while the male drifted attentively around and above her. Finally the female righted herself and rested quietly again a few inches off the bottom. Five hours after the first appearance of the egg raft, the fish still exhibited this courting behavior and the raft had apparently not been extruded to any greater extent. The following morning, ten hours after the initial effort, it was found that the female had succeeded in eliminating the egg mass. Both fish appeared normal and were near the surface of the water in the sargassum weed. The egg raft floated normally and there was no apparent difference in form between the scrolls at either end. Samples from either end of the egg raft were examined microscopically and it was found that, whereas eggs from the one end had reached a blastula stage normal for eggs twelve to fourteen hours after fertilization, the eggs sampled from the opposite end of the raft had not been fertilized, although they appeared fresh and normal. No motile sperm could be seen in these samples. Evidently the male did not fertilize the eggs when they were finally eliminated.

During the day following this fifth spawning, the male became increasingly aggressive towards the female, constantly chasing her about the tank. The fish were finally separated. The female was replaced in the male's tank three days later and a spawning occurred. The male, however, exhibited extremely aggressive behavior. The female later died after being somewhat mutilated about her fins and tail by the male.

Observations on Additional Spawnings

The month following the original spawning observations, a new female was placed in the tank with the male used in the original spawnings. The observations of these spawnings make an interesting supplement to those of the original pair. The production of egg masses was not as frequent as that of the first female; the courting period seemed in general to extend over a longer period than before; and the male did not exhibit the extremely rough behavior described above for the fifth spawning on January 18th.

Egg Laying Cycle: Second Female.—February 20th: An unfertilized egg raft was found in the morning in a tank with a single female.

February 25th, 9:20 p. m. Spawning.

March 3rd, 6:27 p. m. Spawning.

1st Spawning, February 25th.—Three days after the production of the first egg raft by this female, February 20th, the female was introduced to the male's tank. The male showed no aggression whatsoever, but became very attentive immediately. The male's jaw trembled conspicuously whenever he approached the female and occasionally tremors would go through his whole body. He hovered close to the female with all fins spread. The female appeared uninterested and kept her dorsal fin down because the male hovered so close above her. Occasionally the male brushed the female with pectoral and pelvic fins. Whenever the male left the side of the female, it was to swim back and forth displaying his fins. Except for these occasional excursions, the male remained in close contact with the female, his pectoral fins touching hers. Occasionally the male even got astride the female, settling down from above until he came to rest with his ventral fins spread across the female's back. The fish maintained this same behavior throughout the day, the female hanging in the weed and the male hovering about. Late in the afternoon the female was seen on the bottom of the tank, all fins collapsed, with the male on top in contact with her, also with all fins collapsed. The fish remained in this position with very little movement throughout the next two days until the early evening of February 25th. During the day of spawning the female appeared much enlarged, two swellings on either side extending from just behind the pectoral fins to the anus.

In the early evening, about an hour and a half before spawning, the male was observed pushing the female around the tank floor in front of him. The female was so large that she seemed unable to swim in proper balance. The male looked very much as though he were pushing a big ball with his nose. The male would occasionally nip the female in the stomach region. The male's fins were all erected, while the female kept her dorsal fin depressed. Occasionally the female would attempt to push the male away by pushing her pectoral fin against his snout. Several times the male opened his jaws very wide, then trembled afterwards. Occasionally he made a very quick, sharp movement, almost like an avoidance reaction. After a few minutes of rest, the male came up behind the female, appeared to bite at her, and forced her ahead of him out of a protecting shelter of algae-covered rock. He then again began pushing the female about the bottom of the tank, shuddering and butting the female out of corners, appearing to attempt to keep her up off the bottom. An hour before spawning, both fish appeared to be resting on the bottom once more, with all fins collapsed. They exhibited no further activity for at least a half hour. The actual spawning was not witnessed, but shortly afterwards both fish were removed from the spawning tank and they were separated from one another.

2nd Spawning, March 3rd.—The day following the first spawning male and female were put together again in the tank used previously. The male immediately began to hover close to the female, sporadically trembling all over in the same way as observed just before previous spawnings. In addition to this over-all trembling, the male exhibited occasional tremors of his lower jaw alone. The following day both fish remained quiet on the bottom of the tank, the male always in close attendance on the female. There was noticeable swelling again in the female's abdominal region. In the evening, the day before spawning, March 2nd, the female was observed holding herself with her pectoral fins in the weed at the surface with the male hovering underneath. The female's respiration appeared to have increased and her shape was changing; her anal fin appeared almost in the former position of the caudal, forced back by a large bulge above the anus. The skin appeared stretched tight and the female seemed to have difficulty in balancing herself. She released the weed which she had been grasping with one pectoral, but seemed unable to swim down, the caudal part of her body floating as if buoyed. The female was seen to give a wide yawn, similar to those observed in the male. The male appeared very attentive, always hovering close to the female, usually with the top of his head in contact with her belly. Several times the female pushed him away with her pectoral. Occasionally the male gave a wide yawn.

In the early morning on the day of spawning, March 3rd, both fish were very quiet, the female being enlarged even more than previously noted. In the early afternoon, the female was on the bottom, with the male standing head down to one side with all of his fins spread over the top of the female. The two fish marched across the bottom, the female in the lead, the male pushing from behind. The male then stood once more on his head, bending the rest of his body over the female. The male showed a burnished copper color and kept his first dorsal spines stiffly erected, while the female's fins were all collapsed. The male swam at the female and then past her, after which she went to the surface and hung in the weed. The male hovered beneath the female, occasionally trembling. No dilation could be seen in the female's genital pore at this time. In his hovering, the male was in almost constant contact with the female's underside as she hung from the weed, occasionally leaning against her and trembling. The female's respiratory rate was almost double that of the male. The female gave a wide yawn, repeated by the male ten minutes later.

As the afternoon progressed, the male circled the female, in constant contact with her, trembling. He would yawn, then attempt to push the female down from the weed. He frequently brushed her ventral surface with his first dorsal spines. He gave a series of about fifteen yawns, then trembled repeatedly. The fish repeated these actions periodically until spawning.

The female remained just below the surface in the weed for about two hours until immediately before spawning. The male hovered very close, trembling and pushing at her with his pectoral and dorsal fins. During this period, the female showed little change except she had swelled even larger and appeared more nearly round, the anal fin forced into a caudal position, the caudal fin at an angle dorsally. The genital pore became visibly distended, although no papilla was evident. About an hour before spawning, distended and ruptured blood vessels could be seen in the tissue underlying the skin of the female's belly. The entire area of the female's body cavity posterior to the insertion of the pectoral fin was a bright, clear pink. Throughout this period, the male never ceased

pushing and shoving the female with fins and first dorsal spines. He repeatedly nudged the female's belly around the genital pore with his snout.

Two minutes before spawning, the female let go of the weed and the male immediately started shoving from behind, trying to start the march, but the female once again grasped a piece of weed with her pectoral. A minute later she released her hold on the weed and the male followed close behind, appearing to nibble at her belly. The two fish shot to the surface; the eggs were laid and fertilized. The actual spawning was so rapid that it was difficult to see what actually happened but it appeared that the eggs may have been slightly extruded and the male may have taken them in his mouth and with a sharp pull, extracted them.

Immediately after the spawning, both fish swam actively back and forth across the tank with all fins spread. The male appeared somewhat aggressive, but at no time during the courting period or spawning did he exhibit the extremely aggressive behavior observed in his spawnings with the first female. However, the two fish were left together after this spawning and the male became increasingly aggressive until several days later the female was found dead. She was in very bad condition, especially in the area of the caudal peduncle, which appeared to have been bitten repeatedly by the male.

Development of Eggs and Larvae

After every spawning the entire egg raft was removed from the aquarium. In several cases the jelly mass was cut into several pieces and each piece was set in a separate container with a supply of running sea water. The water temperature varied between 21° and 23° C. When the egg raft was cut, the larvae that broke out of the jelly mass hatched several hours earlier than if the raft was left whole. Small numbers of the larvae were put in large aquaria without running water. It was found that they could be kept successfully in this way for about six days, but at this time, when the yolk sac was almost used up, the larvae died.

Development Schedule:

0-2 hours: The egg is extremely transparent, glassy in appearance. It is oval when freshly laid and only becomes spherical at the time of the second cleavage (Plate III, Figures 1, 2). Before the first cleavage, the eggs average 0.7 × 0.6 mm. There is a fine line of lightly granular protoplasm encircling the yolk, concentrated at the animal pole into a thin disc 0.3 mm. in depth.

2 hours: (Plate III, Figure 1). The first cleavage is complete, forming two equal blastomeres as a cap on the pole of the yolk.

3 hours: (Plate III, Figure 2). The second cleavage is complete, and the nuclei are distinct. The egg is now spherical.

4 hours: (Plate III, Figure 3). The third cleavage is complete, forming an eight-cell blastodisc. This blastodisc has spread out over the top of the yolk sphere, and some further cleavage lines may be indistinctly seen at its periphery.

5½-10 hours: There is a thin (probably one-cell thick) layer of cells spreading down over the yolk.

21-27 hours: (Plate III, Figure 4). The egg has reached a late blastula stage and a distinct germ ring can be seen.

27-36 hours: (Plate III, Figure 5). The entire germ ring has thickened considerably, one area expanded more extensively than the remainder, forming the beginning of the embryonic shield.

36-40 hours: (Plate III, Figure 6). The embryonic shield has thickened and lengthened very considerably.

40-48 hours: (Plate III, Figure 7). The neural keel is now apparent as a long band down the center of the embryonic shield. The average length of the embryonic shield at this time is 0.3 mm.

48-65 hours: (Plate III, Figure 8). The formation of the optic vesicle is first noted after the formation of three to four somites. The number of somites increases over a period of twenty-four hours. At the first appearance of the optic vesicle, the jelly mass of the egg raft has lost much of its rigidity, the entire mass softening and swelling and sinking to the bottom of the aquarium (Plate I, Figure 2). The microscopic framework of the jelly is breaking up, the regular octagonal capsule about each larva is becoming irregular and indistinct. The length of the three- to four-somite larvae is 0.5 mm.

65-75 hours: (Plate III, Figure 9). The somites now number thirteen to fifteen and the lens has formed in the optic vesicle. The head curves slightly ventrad around the yolk, the tail bud is free. There is a distinct enlargement dorsal and posterior to the head which appears to be the auditory placode. The total length of the larva is now 0.6 mm.

90-96 hours: Although still enmeshed in the soft jelly of the egg raft, the larvae are very active. The heart is beating strongly, and the tail, free of the yolk, twitches sporadically. Almost none of the capsule framework of the jelly mass remains.

4½ days: (Plate III, Figure 10). The newly hatched larva has a large yolk sac. There is considerable pigmentation; melanophores cover the top of the head and the eyeballs and there is a solid band of melanophores in the dorsal peritoneum across the top of the yolk sac. The pectoral fins are well developed but there is no evidence of the pelvic fins. The total length of the larva has reached 1.4 mm.

11 days: (Plate III, Figure 11). The yolk sac has almost disappeared. The gut has broken through to the exterior, caudad to the yolk sac. The larva is heavily pigmented both in the head region and in the dorsal part of the body cavity. One larva stained with toluidine blue shows that cartilaginous mouth parts are present at this stage. There is still no evidence of the appearance of the pelvic fins and there is no indication of the formation of any fins other than the pectorals. None of the larvae survived this age by more than a day.

Résumé and Discussion

From observations described at length in the foregoing section, the generalized behavior patterns of these fishes during reproduction in small aquaria have been summarized below. Detailed description of each spawning was considered necessary to facilitate the emergence of a concept of the typical behavior pattern together with the range of variation that may be found within this pattern. Once the spawning activities of these fish are established, there is an adequate basis for comparison with the reproductive behavior of other pediculate fishes, of which there is yet no report.

In all the spawnings observed, although the fish fed the night before spawning, neither male nor female made any attempt to feed throughout the courting period which generally lasted about twelve hours. During the courting period, both fish remained mostly inactive, although at the approach of spawning the male became increasingly active, especially in the later spawnings with the first female. There was considerable variation in the colors exhibited by the fish during the spawning periods. Whereas in the first series the female retained the light tan color closely matching the sargassum weed, typical of these fish in captivity, the male became a distinctive dark brown color during the courting period and kept it until the spawning occurred, when he resumed the light tan. However, the second female became almost as dark as the male did during the spawning periods.

Towards the end of the courting period, the typical position of the female was vertical, snout down. As the courting period progresses, the fish's abdomen swells and the center of gravity evidently shifts so that finally the female is standing on her head. The change of the center of gravity probably may be accounted for by the change of position of the eggs resulting from ovulation. The tremendous abdominal distention and the increasing buoyancy of the female previous to spawning seems to suggest that the egg raft may be almost in the same condition as when it is released. Moreover, there was no noticeable expansion or stiffening of the egg mass as it was released. Although it would be very unusual for the chorionic membranes to imbibe fluid while still in the body cavity, there seems to be sufficient indication that this may be the case to warrant further study.

After the extended courting period there is a very short pre-spawning march, during which the two fish position themselves for the amazingly rapid spawning. In the first two spawnings this march appeared definitely related to the bottom, but from observations on subsequent spawnings it is believed that this behavior was a result of the shallowness of the water. Typically, the female proceeds slowly, snout tipped down, with the male swimming in a horizontal position slightly below and behind her with his snout at the level of the female's vent. Then the female may swim to the surface with the male in position behind her, or she may be pushed upwards tail first by the male. In both cases, as the fish approach the surface, the male swings directly away from the female, swimming up past her, breaking the surface of the water, and returning to the bottom, leaving the female at the surface shaking free from the egg raft for several seconds. The duration of spawning is extremely brief, one spawning, which appeared slightly longer than others observed, lasting less than 10 seconds. It appears that as the fish go into the pre-spawning march, the egg raft is in its final form within the cavity of the female, permitting its very rapid release.

Although, because of the extreme speed at which the spawning takes place, it was impossible to see the precise behavior of the fish in the extrusion of the egg raft and fertilization, both direct observations and slow speed and single-

frame study of motion pictures of the spawnings point out the probable sequence of events. In all of the five spawnings of the first series, which were closely watched, the male's path past the female during the spawning appeared very similar, with little variation. From a position below and behind the female, with his snout just at the level of the oviduct pore, the male made a sharp turn of ninety degrees away from the female, and then as he gained a position out from under her, he turned to swim upwards past her. The male appeared to take almost the exact same path as he passed the female following the fifth spawning, which was incomplete, leaving the egg raft only partially extruded from the pore. The male followed the same path observed in the more normal spawnings, but very much more slowly. The manner in which the male turned away from the female with the egg raft in his mouth appeared to correspond closely to the movements in actual spawning. In the opinion of Miss Rasquin, from her observations on the second female, it appeared as though the egg raft might have been slightly extruded and the male have taken it in his mouth and, with a sharp pull, have extracted it. In the slow motion photography of the second spawning, although the detail is not clear, the frames covering the spawning show the female below the surface with the egg raft stretched from her oviduct pore upwards, where the male is splashing the surface (Plate II, Figure 7). Although the male cannot actually be seen, he has been traced to and from this position in the preceding and following frames of the film. It is possible that the egg raft, which is extremely buoyant, merely follows in the male's wake to the surface. However, the activity of the male observed in the fifth spawning of the first series points to the probability of the male taking an active part in stretching out the egg raft from the female's pore.

The successful mating of these fish without one eating the other may be attributed to pairing them when both were in full reproductive condition—which, to a great extent, was a matter of chance. These fish had been kept in individual tanks because of the cannibalism common in this species, and shortly before pairing both females had released egg rafts. Upon introduction of the female to the male's tank, the male displayed none of the aggressive activity usual towards any recent addition to the tank. The first female was left with the male even after spawning and the two lived successfully together. However, with each spawning the male became increasingly aggressive, although there was adequate food available, until the female finally died. The second female was removed from the tank after her first spawning to avoid the aggression of the male, but after the second spawning the two fish were left together. The male did not show aggression during the prespawning period as with the first female, but after spawning he succeeded in killing the second female. In both cases, after the peak of spawning behavior, the male showed an increase of aggression, chasing and biting at the female, although he apparently made no actual attempt to eat her—which is typical behavior for any two of these fish in competition in one aquarium. The female apparently was in an exhausted condition after spawning and never exhibited aggression.

Comparison of the three available schedules of egg raft production, those of the two females reported here and the schedule reported by Breder (1949), suggests some stimulation of the female's egg production by the presence of the male. Intervals between egg production of the lone female reported by Breder range from three to twelve days. However, the first female reported here produced an egg raft regularly every three days over a surprisingly long period. This female was kept constantly with the male through the fifth spawning. At the time of the fifth spawning, the fish were separated for three days and the interval between raft productions was extended to four days. The second female here reported was separated from the male after her first spawning, although replaced with him after just one day. The interval between first and second spawnings here was six days. As this female was much slower than the first, when left with the male after spawning, the male lost his attentive courting attitude and became increasingly aggressive until the female was killed. The success of the long period of consecutive spawnings without separation of the first female was probably due to her ability to produce egg rafts in such rapid succession. Almost immediately after spawning, the male showed courting behavior. The schedule reported by Breder shows the female sargassum fish to be capable of short three-day intervals between egg mass production, but there was no male present and the intervals generally were much longer.

The development of the *Histrio* egg is relatively slow. When they are kept at a temperature varying from 21° to 23° C., the larvae hatch after four to five days. As development proceeds, the firm, gelatinous membranes of the egg raft deteriorate so that just before hatching the larvae are enmeshed in a loose framework which is all that remains of the chorionic membranes. For about six days following hatching the larvae drifted about the aquarium just be-

neath the surface film. The pectoral fins were well developed but the pelvic fins had not yet appeared. As with many of the pelagic fishes in captivity, none of the larvae survived after six or seven days following hatching, the time at which the yolk sac was used up and the larvae should have begun to feed.

The behavior pattern described for these fishes observed in aquaria must obviously be considered as modified from that occurring under natural conditions in beds of sargasso weed drifting in the open sea. Physical differences alone in the two environments would suggest modifications in the various phases of behavior. Normally only a single adult fish is found in one clump of weed, which circumstance can be related to the voracious appetite of *Histrio*. In this case, if they follow a pattern typical of many animals, it can be presumed that when the males reach the peak of sexual development they make expeditions into neighboring clumps of weed in search of a receptive female. On finding a gravid female, the courting behavior of the two fish would be substantially the same as observed in aquaria, including color change and relative quiescence until spawning. There are evidently no reports of the collection from one clump of weed of two fish, one differing markedly in color from the other. This question should be studied by means of field work.

The relative inactivity of the fish during courtship would be qualified by the constant motion of the sargasso weed in open sea. The male could be expected to move continuously, following the shifting position of the female. In deep water there would be no firmly anchored rocks or shells as on the bottom of the aquarium, but rather the lower mass of a weed clump. Under these conditions, it can be presumed that the well-defined pre-spawning march of these fish is considerably modified and probably consists only of the male shifting in the rolling weed until he can position himself for the dash to the surface with the female. Since there are such short intervals between these spawnings, it is probable that once the pair is established, the two fish remain together until one or the other is exhausted.

Although in aquaria the egg raft sinks to the bottom while the majority of the larvae still remain enmeshed in the partially deteriorated membranes, it is hardly conceivable that this should be the case in natural conditions. In the open sea even the mildest wave action would be expected to loosen the softened raft even further, allowing the larvae to break free while the raft was still at or just below the surface.

Summary

1. From the spawnings here recorded and records in the literature of egg rafts produced by *Histrio histrio*, no specific spawning season has yet been defined.
2. The typical form of the egg raft produced by the sargassum fish is described.
3. An egg raft released by an *Antennarius multiocellatus* is described and compared with that of *Histrio*.
4. The microscopic structure of the egg rafts of both *Histrio* and *Antennarius* is described and compared to that of *Lophius piscatorius*.
5. The usual aggressive attitude of both sexes is evidently fully suppressed with the maturing of the gonads.
6. Schedules of the females' egg production are given, and courting and spawning behavior is described.
7. The progress of the *Histrio* egg and larva is described through eleven days of development.

Bibliography

Barbour, T.
1942. The northwestern Atlantic species of frog fishes. Proc. New England Zool. Club, 19: 21-40.

Breder, C. M., Jr.
1949. On the relationship of social behavior to pigmentation in tropical shore fishes. Amer. Mus. Nat. Hist. Bull., 94: 94-95.

Gill, T.
1908. Angler fishes, their kinds and ways. Smithsonian Rept. for 1908, 607-9.

Gudger, E. W.
1937. Sargasso weed fish "nests", made by flying fishes not by sargasso fishes (Antennariids): A historical survey. Amer. Naturalist, 71: 363-81.

Hornell, J.
1921. The Madras marine aquarium. Madras Fisheries Bull., 14: 80.

Nelson, O. E.
1953. Comparative embryology of the vertebrates. The Blakiston Company, Inc., New York: 165.

Proctor, William, et al.
1928. Fishes—a contribution to the life-history of the angler, (*Lophius piscatorius*). Biol. Survey of the Mount Desert Region, Corfield, Bar Harbor, Maine.

RYDER, J. A.
1887. On the development of osseous fishes, including marine and freshwater forms. U. S. Comm. of Fish and Fisheries, Part XIII, Rept. Commissioner for 1885, Appendix: 518.

EXPLANATION OF THE PLATES

PLATE I. *Histrio* Egg Raft; *Antennarius* female and egg raft. All ⅓ life size.

FIG. 1. Fresh *Histrio* egg raft.

FIG. 2. *Histrio* egg raft 3 days old. Sinking, swelling, and beginning to break up just prior to hatching.

FIG. 3. *Antennarius* female with abdomen much enlarged just prior to elimination of egg raft.

FIG. 4. *Antennarius* egg raft.

FIG. 5. *Antennarius* female just following egg laying.

PLATE II. Courting Behavior.

FIG. 1. Typical courting position: the female tipped snout down behind weeds with just the tail showing, the male hovering above her.

FIG. 2. Typical pre-spawning position: the female below, the male above making a short turn without losing contact.

FIG. 3. Advanced courting: the male on the left trying to force his way into position for the pre-spawning march, the female on the right pushing at the male with pectoral fin.

FIG. 4. Extremely aggressive behavior of the male exhibited in the fifth spawning. The male on the left shaking the female by the tail.

Spawning Sequence from Selected Motion Picture Frames

FIG. 5. Beginning of the pre-spawning march: the male below with snout closely applied to female's vent.

FIG. 6. The male below pushing the female towards the surface with his snout.

FIG. 7. Female below with the egg raft still attached stretched to the surface; the male splashing the surface.

FIG. 8. Male on the bottom, female shaking free of the egg raft.

FIG. 9. Male and female both on bottom, egg raft taking shape at the surface above them.

PLATE III. *Histrio* Eggs and Larvae. Photographed alive except 11-day larva. 42 × except as otherwise noted.

FIG. 1. 2 hours. 2-cell.

FIG. 2. 3 hours. 4-cell.

FIG. 3. 4 hours. 8-cell.

FIG. 4. 21-27 hours. Early germ ring.

FIG. 5. 27-36 hours. Beginning of embryonic shield.

FIG. 6. 36-40 hours. Embryonic shield extended.

FIG. 7. 40-48 hours. Late embryonic shield with neural keel.

FIG. 8. 50-65 hours. 8 somites.

FIG. 9. 65-75 hours. 13-15 somites, optic vesicle with lens, tail free.

FIG. 10. 4½ days. Large yolk sac, melanophores across top of yolk and in head region. 34×.

FIG. 11. 11 days. Yolk sac much reduced, vent broken through, mouth parts formed. Stained and mounted. 34×.

FIG. 1

FIG. 3

FIG. 2

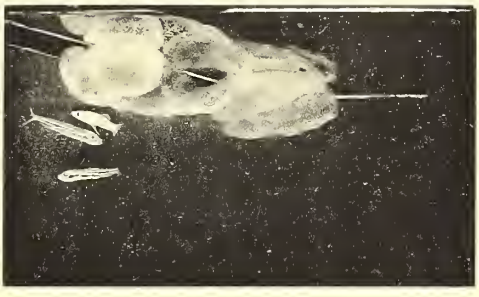

FIG. 4

FIG. 5

OBSERVATIONS ON THE SPAWNING BEHAVIOR AND THE EARLY LARVAL DEVELOPMENT OF THE SARGASSUM FISH, HISTRIO HISTRIO (LINNAEUS)

FIG. 1

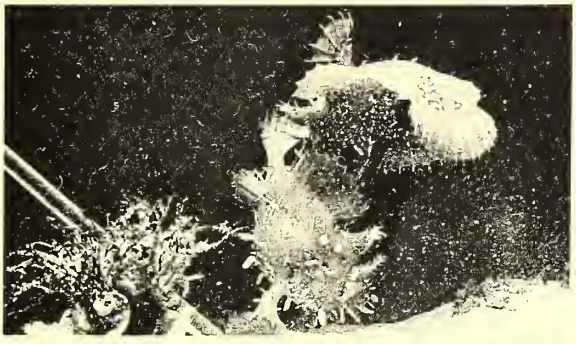

FIG. 2

FIG. 3

FIG. 4

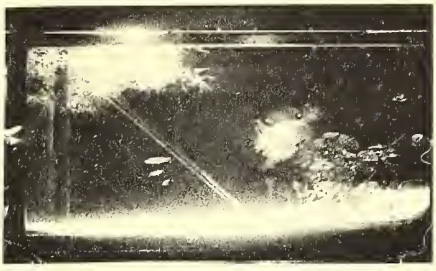

FIG. 5

FIG. 6

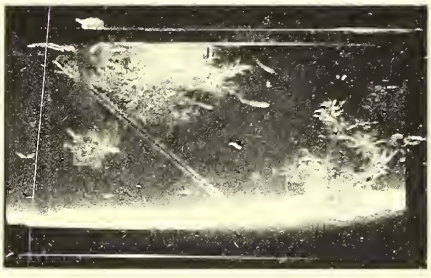

FIG. 7

FIG. 8

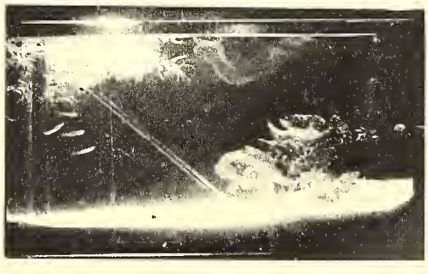

FIG. 9

OBSERVATIONS ON THE SPAWNING BEHAVIOR AND THE EARLY LARVAL DEVELOPMENT OF THE SARGASSUM FISH, HISTRIO HISTRIO (LINNAEUS)

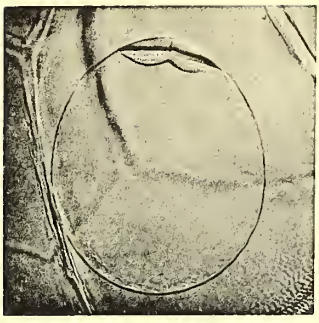

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

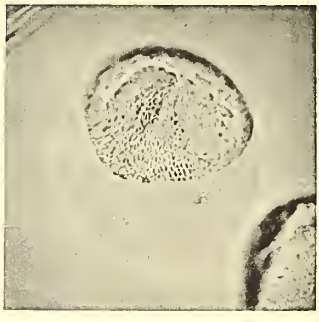

FIG. 6

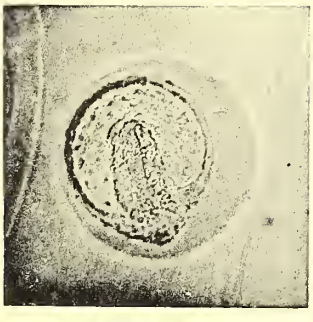

FIG. 7

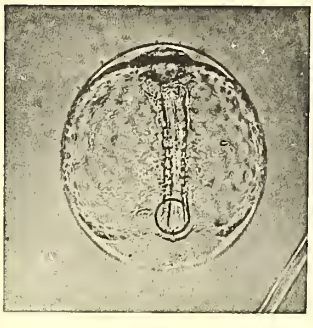

FIG. 8

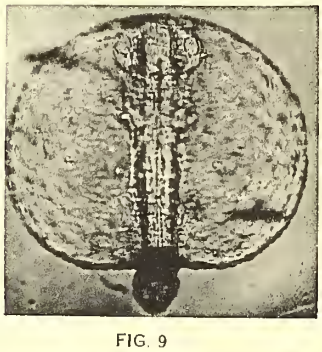

FIG. 9

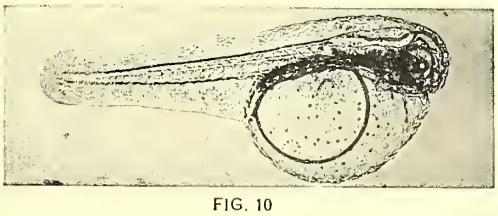

FIG. 10

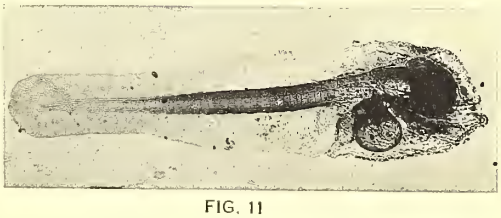

FIG. 11

OBSERVATIONS ON THE SPAWNING BEHAVIOR AND THE EARLY LARVAL DEVELOPMENT OF THE SARGASSUM FISH, HISTRIO HISTRIO (LINNAEUS)

13

A Revision of the Genus *Eubaphe* (Lepidoptera: Geometridae)

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(Text-figures 1-43; Plates I & II, Figures 44-63)

In the course of examining a number of genitalia preparations of male specimens of the larentiid genus *Eudule* it became apparent that the genus, as at present understood, contained species belonging to three distinct groups, which can be conveniently referred to the following three available genera; *Eudule*, *Eudulophasia* and *Eubaphe*. Examination of the genitalia of the females confirmed this division. The characters of the three genera are set out in the present paper, together with a revision of the species included in the genus *Eubaphe*.

Eudule Hübner

Eudule Hübner, 1823, Zuträge z. exot. Schmett., 2:14.
Type species: *Eudule pulchricolora* Hübner.

Mennis Walker, 1854, List Lep. Ins. B. M., 2:379.
Type species: *Mennis orislinea* Walker.

Diplochroa Herrich-Schäffer, 1856, Samml. aussereurop. Schmett., 1:33, 50.
Type species: *Diplochroa bicentraria* Herrich-Schäffer.

Polyomma Staudinger, 1894, Iris, 7:98.
Type species: *Polyomma phlaearia* Staudinger.

The male genitalia of *Eudule* are characterized by a concave saccus and a strongly sclerotized arched uncus, the broad apex of which is sometimes incised; the costal margin of the valve has a strongly sclerotized arm arising from the base; the distal margin is also sclerotized and is sometimes produced beyond the apex of the valve. The aedeagus is stout and shorter in length than the valve and the vesica bears strongly sclerotized cornuti. The eighth sternum is weakly sclerotized and bears a pair of deciduous hair-tufts. In the genitalia of those females so far examined, the ostium bursae is strongly sclerotized and wrinkled and the ductus bursae is often extremely short.

Superficially *Eudule* falls into two sections. One, listed below as typical *Eudule*, has the costa of the forewing considerably longer than the inner margin and in the male the abdomen is long, projecting beyond the anal angle of the hindwing. In the section for which the name *Polyomma* is available, the costa and the inner margin are almost equal in length and the abdomen in the male does not project beyond the anal angle of the hindwing.

Section *Eudule*

trichoptera Perty, 1833
  hesperina Burmeister, 1878 (part)
  costata Warren, 1897
schausi Dognin, 1922
venata Schaus, 1892
  strigilis Druce, 1896
sororcula Schaus, 1929
allegra Schaus, 1929
una Schaus, 1892
striata Druce, 1898
amica Druce, 1898
  rufithorax Warren, 1905
pulchricolora Hübner, 1823
  semele Burmeister, 1878. Syn. nov.
  plurinotata Warren, 1905.
orislinea Walker, 1854
bimacula Walker, 1854
  bicentraria Herrich-Schäffer, 1856
  aperta Warren, 1901
halia Druce, 1885
retroacta Prout, 1934
herona Druce, 1885
atrimorsa Dognin, 1902
parca Warren, 1906
hagno Druce, 1885
ficulnea Druce, 1885
  f. *albifera* Prout, 1918
fidentia Druce, 1885
limbata Burmeister, 1878
  cytherea Schaus, 1892
sceata Schaus, 1892
orilochia Druce, 1885
austria Maassen, 1890
  ithrites Druce, 1901
  malefida Warren, 1904
  simulans Warren, 1904

basipuncta Warren, 1906
annuligera Warren, 1905
arctiata Warren, 1904
leopardina Druce, 1896

SECTION *Polyomma*

ficaria Druce, 1885
f. *semirubra* Dognin, 1902
pyristacta Prout, 1929
ockendeni Warren, 1907
phlaearia Staudinger, 1894
flavinota Warren, 1904
f. *nigrata* Warren, 1905
venitorta Dognin, 1910
lucigerata Walker, 1863
ambiguata Snellen, 1874
aluta Felder, 1875
reversa Dognin, 1913
albata Warren, 1897
costigutta Dognin, 1911
aperta Dognin, 1913
secticolor Prout, 1931

EUDULOPHASIA Warren

Eudulophasia Warren, 1897, Novit. zool., 4: 456.
Type species: *Ameria invaria* Walker.

The genus *Eudulophasia* differs from *Eudule* in having an evenly rounded saccus and a long tapered uncus. The sclerotized arm arises from half way along the costal margin and not from the base and the distal margin of the valve is membranous. The aedeagus is slender and shorter in length than the valve and the vesica is weakly ornamented. The eighth sternum bears a pair of broad-based and apically tapered rods. In the female genitalia the ostium bursae is weak; the membranous ductus bursae is long and slender and the bursa copulatrix is ornamented with minute teeth.

The species in the genus are short bodied and in color capucine orange to orange chrome with a slender black margin to both wings, the single exception being *heterochroa* Felder, which has uniformly black hindwings. Listed below is a provisional arrangement of the species referred to *Eudulophasia.*

invaria Walker, 1854
moeschleri Kirby, 1892
aurora Burmeister, 1878
nigricosta H. Edwards, 1884
circumducta Warren, 1900
f. *latiorata* Warren, 1905
sanguinea Butler, 1877
sicelides Druce, 1885
heterochroa Felder, 1875
seminigra Warren, 1904

EUBAPHE Hübner

Eubaphe Hübner, 1823, Zuträge z. exot. Schmett., 2:20.
Type species: *Eubaphe lobula* Hübner.

Ameria Walker, 1854, List Lep. Ins. B. M., 2:554.
Type species: *Ameria conformis* Walker.

Euphanessa Packard, 1864, Proc. ent. Soc. Philadelphia, 3:102.
Type species: *Nudaria mendica* Walker.

Leptidule Butler, 1877, Trans. ent. Soc. Lond., 10:368, pl. 8:11.
Type species: *Ameria integra* Walker.

In *Eubaphe* the male genitalia have a broadly rounded saccus and a weak, arched uncus; socii are sometimes present. The costal margin of the valve is developed as in *Eudulophasia;* the distal margin is membranous and often incised at or about midway. The aedeagus is slender and greater in length than the valve. The seventh and eighth sterna are strongly developed; the seventh sternum consists of a pair of sclerotized rods, the anterior halves usually fused and the posterior halves angled or bowed outwards and connected by slender membranes or fused to the two processes on the eighth sternum, which are broad-based and tapered posteriorly. In most species there is a pair of hair-tufts, which are secreted within the body wall and are extrusile just beyond the posterior edge of the sixth sternum. In most of the species also, the fifth and sixth sterna each bears a slender rod-like process, which arises internally from the center of the anterior edge and extends anteriorly, that from the sixth sternum being longer.

The species included in the genus *Eubaphe* fall into three sections. In the male genitalia of Section A the distal margin of the valve is not incised, the sclerotized processes on the seventh and eighth sterna are fused, neither the fifth nor the sixth sternum bears a process on its anterior edge and the socii are wanting; in the female genitalia the ductus bursae is strongly sclerotized and is at least as long as the diameter of the bursa copulatrix, which is globular and spined but not sclerotized. To this section belong the species *rhotana, eulathes, lineata, helveta, pauper, bada, daxata* and *tripunctata.*

In Section B the males differ from those of the preceding section in the sclerotized processes of the seventh and eighth sterna, which are hinged and not fused; the distal margin of the valve may be simple or incised; socii are present in *conformis* only. The females of the section are characterized by the strongly developed processes on the ostium bursae; the bursa copulatrix is longer than broad, partially sclerotized and heavily ornamented with spines. To this section belong the species *meridiana, mendica, pumilata, unicolor* and *conformis.*

In the male genitalia of Section C the distal margin of the valve is incised, socii are present in all species except *weyenberghii,* the sclerotized processes on the seventh and eighth sterna are hinged, except in *orfilai,* and the anterior of the fifth and sixth sterna bear slender rod-

like processes. In the female the ductus bursae is strongly sclerotized but simple; the bursa copulatrix varies in shape, is sclerotized in part and heavily ornamented with spines. To this section belong the species *deceptata, fieldi, cupraria, hesperina, integra, orfilai, weyenberghii, aeetes, medea* and *lobula.*

In superficial appearance, too, the genus *Eubaphe* appears to form a natural group; the ground color of the wings in all but five species is orange buff to capucine orange, that of the exceptions being white to pale straw. Six species, distributed through the three sections of the genus, are patterned with hyaline spots; five species have a small amount of black marking, principally on the veins, and the remaining species are unpatterned, except the males of four species, which have sex marks on the wings.

The neuration of the species in the genus is somewhat variable and Text-fig. 1 shows that of *Eubaphe mendica,* which is common to twenty of the twenty-three species and also common to the type species of the other two genera, *Eudule* and *Eudulophasia.* The three exceptions to this pattern are *lobula,* in which vein *Sc*1 of the forewing arises from close to the distal angle of the areole, near the stalk of veins *Sc*2-5; *medea,* in which vein *Sc*2 of the forewing arises from three-quarters of the upper surface of the areole; and the third is *integra,* in which veins *Sc* and *M*1 of the hindwing arise separately from the cell.

The structure of the antennae, palpi and legs appears to be identical in all three genera.

Eubaphe is most richly represented in Central America, where over half the known species occur. The distribution of the genus extends southward to Paraguay and the Argentine, eastward to Cuba and northward into the United States and southern Canada.

The name *Eubaphe* has hitherto been widely used in the subfamily Arctiinae, but when the genus was first published it was monobasic, containing the single species *Eubaphe lobula* Hübner; *Eubaphe aurantiaca* Hübner, which is usually cited as the type species, was not added to the genus until a later date (1827-1831, Zuträge z. exot.Schmett., 3:9). The next valid name to replace *Eubaphe* in the Arctiinae appears to be *Holomelina* (Herrich-Schäffer,1856, Samml. aussereurop. Schmett., 1:15, 17), the type species of which is *Eubaphe aurantiaca* Hübner.

The color names used are taken from Ridgway's "Color Standards and Color Nomenclature," the sole exceptions being those quoted from original descriptions.

The magnification of the figures of the terminal sterna is ×28; that of the aedeagi is ×42.

I should like here to express my thanks to Dr. Orfila of the Argentine Museum of Natural History for photographs of the Burmeister types and for the loan of the abdomens of the types; also to Mr. W. D. Field of the United States National Museum for his ready help in comparing British Museum material with the types of the Dognin, Schaus, Dyar and Warren species in that institution.

SECTION A

Eubaphe rhotana (Druce)

(Text-figs. 5 & 11; Plate fig. 46)

Eudule rhotana Druce, 1894. Ann. Mag. nat. Hist., (6) 13:178.

Eudule rhotana Druce, 1897, Biol. Cent. Amer., Zool., Lep. Het., 2:403, pl. 78:18.

Palpi, antennae and legs, except mid and hind coxae, fuscous. Head, thorax, abdomen, mid and hind coxae pale maize yellow. Wings semi-hyaline, pale maize yellow; the costa, distal and inner margins orange buff; the veins on both wings are finely marked with fuscous.

Genitalia. Male.—Uncus evenly rounded; socii wanting. Distal edge of valve evenly curved to a rounded apex. Apex of aedeagus lightly scobinate; basal third narrowed with the extremity rounded. There are no cornuti. The seventh and eighth sterna are as shown in the figure.

Female.—Ductus bursae strongly sclerotized, the posterior edge deeply concave; it is ribbed longitudinally, narrowed anteriorly and is slightly longer than the diameter of the bursa copulatrix, which is globular and membranous with three signa, each a cluster of spines narrowly connected by a slender band of spines. The whole ornamentation extends for two-thirds of the longitudinal circumference.

The type is in the British Museum.

Distribution.—Mexico, Guerrero.

Eubaphe eulathes (Dyar)

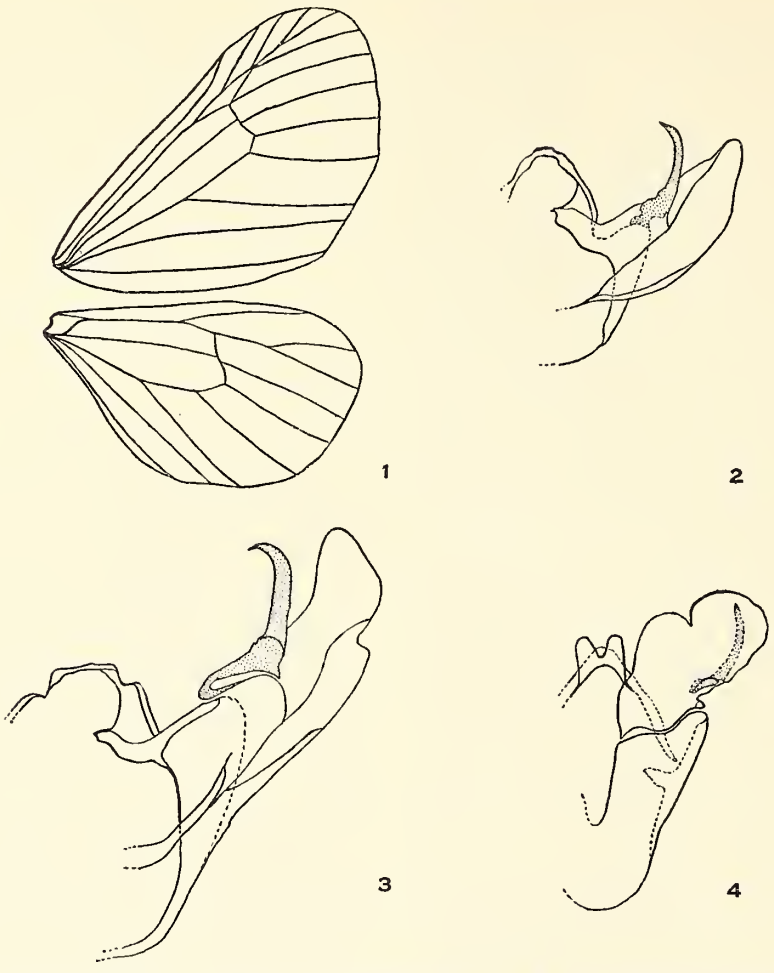
(Text-figs. 7 & 12; Plate fig. 44)

Eudule eulathes Dyar, 1920, Insec. Inscit. menstr., 8:195.

"White; costa brownish yellow from base to end of cell; veins on both wings narrowly lined in black, less distinctly on hind wings. Expanse 25 mm."

Genitalia. Male.—Differs from *rhotana,* to which it is closely related, in the shape of the seventh and eighth sterna, as will be seen from the figure; it differs also in the aedeagus, which is narrowed at one-half instead of one-third and is lightly sclerotized and ribbed apicad.

Female.—Differs from *rhotana* in the shape of the posterior edge of the ductus bursae, which is broadly and very shallowly v-shaped.



TEXT-FIGS. 1-4. 1. *Eubaphe mendica,* Neuration. 2. *E. lineata,* Valve. 3. *E. mendica,* Valve 4. *E. cupraria,* Valve.

The type is in the U. S. National Museum.

Distribution.—Mexico, Zacualpan.

EUBAPHE LINEATA (Druce)

(Text-figs. 2, 6, 13; Plate fig. 45)

Eudule lineata Druce, 1885, Biol. Cent. Amer., Zool., Lep. Het., 1:138, pl. 13:11.

Palpi, antennae and legs fuscous. Thorax and abdomen orange buff to capucine orange. Wings capucine orange to pale pinkish-buff; on the forewing the lower median, veins *M*1-3, *Cu*1-2 and *A*2 are strongly marked in fuscous; the tips of the radial veins are sometimes similarly marked.

Genitalia. Male.—Uncus narrowly arched; socii wanting. Distal margin of valve evenly curved to a narrowly rounded apex. Aedeagus with two small scobinate patches, one ventrad and the other dorsad of the apex. Vesica with two cornuti; one is slender and tapered with a fold basad and is one-half as long as the aedeagus; the second is short, stout and weakly sclerotized and equal in length to the width of the aedeagus. Seventh and eighth sterna as shown in the figure.

Female.—Ductus bursae strongly sclerotized, ribbed longitudinally and narrowed anteriorly; it is equal in length to the diameter of the bursa copulatrix, which is membranous with a band of spining, extending laterally for two-thirds of the circumference, connecting the two signa, each of which is a cluster of spines.

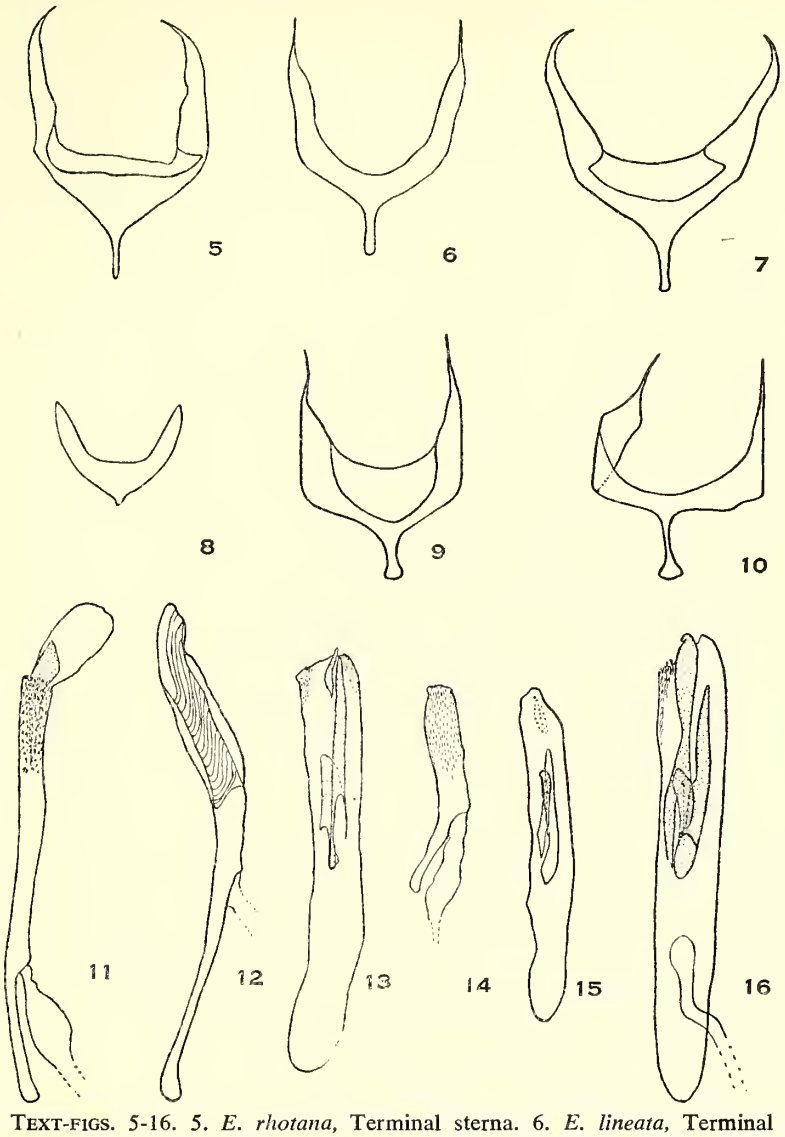
The type is in the British Museum.

Distribution.—Guatemala, Calderas, Quiche Mtns. and Totonicapam.

EUBAPHE HELVETA (Barnes & McDunnough)

(Text-figs. 8, 14)

Eudula helveta Barnes & McDunnough, 1907, Canad. Ent., 39:98.



TEXT-FIGS. 5-16. 5. *E. rhotana,* Terminal sterna. 6. *E. lineata,* Terminal sterna. 7. *E. eulathes,* Terminal sterna. 8. *E. helveta,* Terminal sterna. 9. *E. pauper,* Terminal sterna. 10. *E. tripunctata,* Terminal sterna. 11. *E. rhotana,* Aedeagus. 12. *E. eulathes,* Aedeagus 13. *E. lineata,* Aedeagus. 14. *E. helveta,* Aedeagus. 15. *E. pauper,* Aedeagus. 16. *E. tripunctata,* Aedeagus.

Eudule helveta Barnes & McDunnough, 1912, Contrib. nat. Hist. Lep. N. Amer., 1(4):31, pl. 14:9.

Closely related to *bada* Druce but differing in the color and pattern; *bada* is capucine orange and *helveta* is orange buff; the antemedial spots and the postmedial fascia in *bada* are little broader than the width of the abdomen; in *helveta* the corresponding markings are at least twice as broad.

Genitalia. Male.—Uncus arched; socii wanting. Valve slender, not incised, the apex narrowly rounded. Costal process arising from the valve at one-third, slender and tapered. Aedeagus lightly scobinate at apex; the width of the basal third is reduced to one-half of that of the remainder. There are no cornuti. The terminal sterna are as shown in the figure.

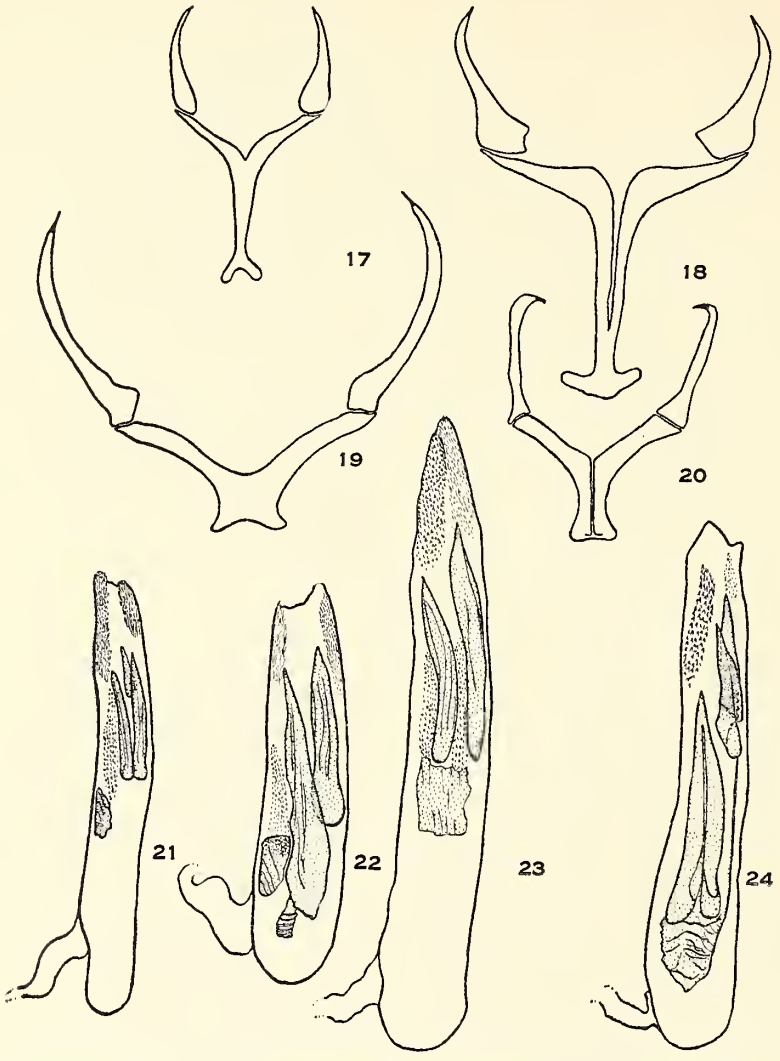
I have so far seen only males of this species. The type is in the U. S. National Museum.

Distribution.—Texas.

EUBAPHE PAUPER (Schaus)

(Text-figs. 9, 15)

Euphanessa pauper Schaus, 1889, Ent. Amer., 5:192.



TEXT-FIGS. 17-24. 17. *E. meridiana,* Terminal sterna. 18. *E. mendica,* Terminal sterna. 19. *E. conformis,* Terminal sterna. 20. *E. unicolor,* Terminal sterna. 21. *E. meridiana,* Aedeagus. 22. *E. unicolor,* Aedeagus. 23. *E. mendica,* Aedeagus. 24. *E. conformis,* Aedeagus.

Euphanessa pauper Druce, 1897, Biol. Cent. Amer., Zool., Lep. Het., 2:403, pl. 78:20.

♂ 28 mm. Considerably larger than *bada* Druce, which has a wingspan of only 21 mm; similar in color and pattern, but the latter is more clearly defined in *pauper* than in *bada.*

Genitalia.—Similar to those of *tripunctata* Druce but differing in the vesica, which bears three weakly sclerotized cornuti, one one-half and two one-quarter as long as the aedeagus. The seventh and eighth sterna are as shown in the figure. I have so far seen only males of this species.

In view of the extensive speciation of *Eubaphe* in Central and South America, it might be better, until more material is available for study, to regard *pauper* and *bada* as distinct species rather than follow the synonymy of Druce.

The type is in the U. S. National Museum.

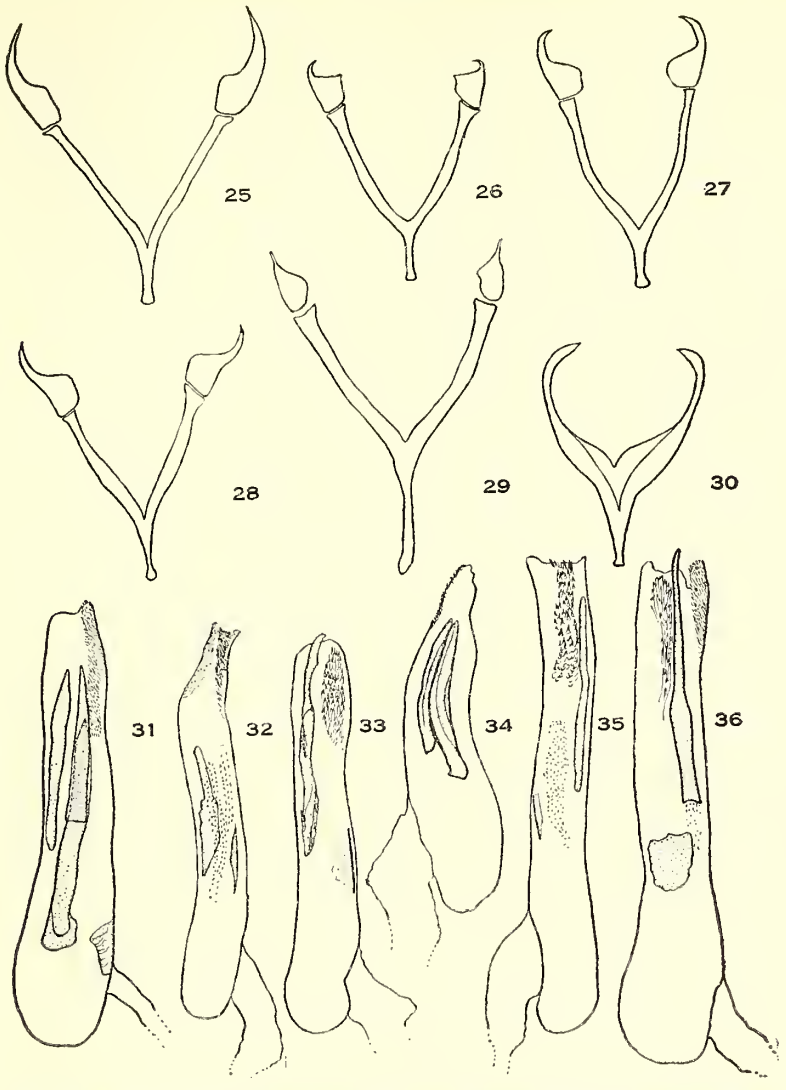
Distribution.—Mexico, Vera Cruz and Hidalgo.

EUBAPHE BADA (Druce)

(Plate fig. 50)

Eudule bada Druce, 1890, Ann. Mag. nat. Hist., (6) 5:215.

♀ 21 mm. Antennae fuscous. Head, thorax and abdomen orange buff. Wings orange buff, slightly hyaline. On the forewing there are two



TEXT-FIGS. 25-36. 25. *E. deceptata,* Terminal sterna. 26. *E. cupraria,* Terminal sterna. 27. *E. fieldi,* Terminal sterna. 28. *E. hesperina,* Terminal sterna. 29. *E. integra,* Terminal sterna. 30. *E. orfilai,* Terminal sterna. 31. *E. deceptata,* Aedeagus. 32. *E. fieldi,* Aedeagus. 33. *E. cupraria,* Aedeagus. 34. *E. orfilai,* Aedeagus. 35. *E. integra,* Aedeagus. 36. *E. hesperina,* Aedeagus.

large, dark antemedial spots, separated by the lower median vein; there is also a broad, dark postmedial fascia extending from just below the costa to vein *A*2, with two distal projections, one along vein *M*1 and the other between veins *M*3 and *Cu*1.

Genitalia.—Ductus bursae strongly sclerotized, bilobed posteriorly and tapered anteriorly to a little more than one-third of the posterior width. Bursa copulatrix ovate and membranous with a narrow band of spines extending half way round the circumference.

Of this species I have seen only the unique female type, which is in the British Museum.

Distribution.—Mexico, V. Ixtaccihuatl.

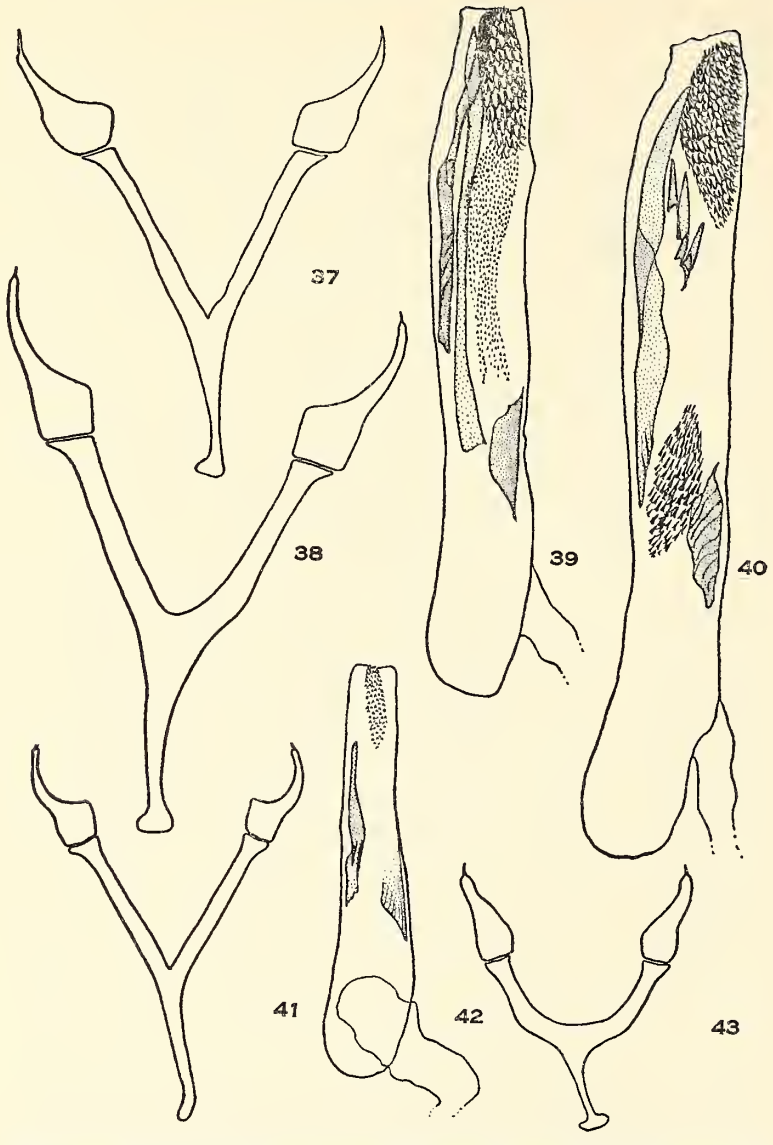
EUBAPHE DAXATA (Druce)

(Plate fig. 47)

Eudule daxata Druce, 1894, Ann. Mag. nat. Hist., (6) 13:177.

Eudule daxata Druce, 1897, Biol. Cent. Amer., Zool., Lep. Het., 2:403, pl. 78:17.

Similar in coloration to *tripunctata* Druce but differing in the black pattern of the forewing and in having the face black. The basal quarter of the costa is narrowly black with a small dot



TEXT-FIGS. 37-43. 37. *E. weyenberghii,* Terminal sterna. 38. *E. medea,* Terminal sterna. 39. *E. weyenberghii,* Aedeagus. 40. *E. medea,* Aedeagus. 41. *E. aeetes,* Terminal sterna. 42. *E. lobula,* Aedeagus. 43. *E. lobula,* Terminal sterna.

at the inner extremity. A slender postmedial fascia extends from two-thirds of the costa to just beyond vein *Cu*2; it is outwardly bowed at the middle; the apical margin is similar to that of *tripunctata.* Vein *M*3 from the distal margin to the postmedial, the middle of the lower median vein and the basal half of vein *A*2 are broadly black.

Genitalia. Female.—Ductus bursae strongly sclerotized, almost twice as long as broad, anteriorly a little narrower than posteriorly; the posterior edge of the dorsal surface is bilobed. The bursa copulatrix is almost globular and is membranous with three signa, each a cluster of small spines, along the lateral circumference connected by a band of minute spines.

Of this species I have seen only the unique female type, which is in the British Museum.

Distribution.—Mexico, Guerrero.

EUBAPHE TRIPUNCTATA (Druce)

(Text-figs. 10, 16; Plate fig. 49)

Eudule tripunctata Druce, 1885, Biol. Cent. Amer., Zool., Lep. Het., 1:138, pl. 13:10.

Palpi and antennae fuscous black; tibiae and tarsi fuscous. Head, thorax, abdomen and hindwing capucine orange. Forewing capucine orange with black markings. There is a minute black spot at the base of the costa and two larger costal spots, one at one-third and the other at two-thirds; there is a narrow marginal line from three-quarters costa round the apex to vein *M1*; vein *M3* is broadly outlined, the markings sometimes extending along the median.

Genitalia. Male.—Similar to *lineata* but differs in the aedeagus and the seventh and eighth sterna. The vesica bears two cornuti, each one-half as long as the aedeagus; one is tapered with a fold basad; the second has a hooked apex. The terminal sterna are as shown in the figure.

Female.—Ductus bursae strongly sclerotized, the dorsal surface produced as two lobes, their posterior edges truncate; it is two-and-a-half times as broad posteriorly as anteriorly and a little longer than the diameter of the bursa copulatrix, which is ovate and membranous with two signa, each a cluster of spines narrowly connected by a band of similar spines.

The type is in the British Museum.

Distribution.—Mexico, Jalapa and Las Vigas.

SECTION B

Eubaphe mendica (Walker)

(Text-figs. 1, 3, 18, 23; Plate fig. 51)

Nudaria mendica Walker, 1854, List Lep. Ins. B. M., 2:576.

Eudule biseriata Herrich-Schäffer, 1855, Samml. aussereurop. Schmett., 1, pl. 76:441.

Antennae, head, thorax and abdomen straw yellow. Legs straw yellow, the femur of the foreleg and the fore- and mid-tibiae are striped longitudinally with fuscous. Wings slightly hyaline, usually straw yellow but sometimes rather paler and sometimes the margins are darkened to orange buff. The forewings are crossed by two transverse fasciae, which extend from just below the costa to the inner margin, an antemedial and a postmedial, each consisting of irregularly shaped fuscous spots lying between the veins. There is an additional fuscous subterminal spot between veins *M3* and *Cu1*.

Genitalia. Male.—Uncus broad and truncate; socii wanting. The apical quarter of the valve is two-thirds as broad as the remainder. The aedeagus has two lightly scobinate patches, one ventrad and one dorsad of the apex. The vesica bears two slender, tapered cornuti, each one-third as long as the aedeagus, and a small sclerotized patch basad of them. Terminal sterna as shown in the figure.

Female.—From the ostium bursae there arise two large, weakly sclerotized processes, posteriorly as broad as the bursa copulatrix, the anterior two-thirds sharply tapered. Bursa copulatrix lightly sclerotized with considerable spining, as shown in the figure.

Of the early stages Forbes writes "larva on violet, also reared by McDunnough on maple; very slender, taking odd positions in life; smooth, green, dusted lightly with brown and marked on sides with red and white."

The type of *mendica* is in the British Museum; that of *biseriata* is not in the Herrich-Schäffer collection in the Zoological Museum, Berlin, and has not yet been located.

Distribution.—Canada, Br. Columbia and Ontario to Nova Scotia; United States of America.

Eubaphe meridiana (Slosson)

(Text-figs. 17, 21; Plate fig. 48)

Euphanessa meridiana Slosson, 1889, Ent. Amer., 5:7.

Similar in pattern to *mendica;* the spots comprising the tranverse fasciae are much smaller and the extra subterminal spot is wanting. In coloration rather deeper toned than *mendica* and much smaller in size, the average wingspan being 20 mm whilst that of *mendica* is 30 mm.

Genitalia. Male.—Uncus broadly rounded. Valve much narrowed and tapered in apical third; apex narrowly rounded. Aedeagus with two lightly scobinate patches, one ventrad and one dorsad of the apex. The vesica bears three cornuti, all tapered spines; two are one-third and one is one-tenth as long as the aedeagus; in addition there is a small sclerotized patch midway along one side. The terminal sterna are as shown in the figure.

Female.—Ostium bursae bearing structures similar to those of *mendica* but they are less distended posteriorly and are evenly tapered anteriorly. The differences in the spining can be seen from the figures.

The holotype ♀ and the two paratypes ♂, labelled Florida, are in the American Museum of Natural History, New York.

Distribution.—Atlantic Coast of the United States of America, from New York to Florida.

Eubaphe rotundata (Cassino & Swett)

Eudule rotundata Cassino & Swett, 1922, The Lepidopterist, 3:150.

"This new *Eudule* is readily distinguished from *helveta* Barnes and *meridiana* Slosson. The color of the superiors is gamboge, like the two species mentioned, being slightly darker on the costa. The inferiors are concolorous with the superiors. The superiors are rounded at the apex. The distance from the center of the costa

to the outer angle is much longer than in *helveta* and *meridiana*, giving the wings a short, stubby, rounded appearance. On the inferiors the length from the base to the center of the outer margin is about one-third more than from the outer angle to the middle of the costal edge. Thus the inferiors are more nearly rounded than in the other species in the genus.

"Head, thorax and antennae are concolorous with the base of the superiors; abdomen lighter. There are three small spots between the veins one-fourth in from the outer edge and one slightly larger spot in the cell. There is a small spot near the inner margin and nearer the base. There are slight indications of a fourth spot near the costa in line with the three spots parallel with the outer margin. The short rotundate appearance of the wings, as compared with the other species, easily distinguishes it.

"Expanse 20 mm."

The original description is quoted, for the species is not represented in the British Museum. The type, from St. George, Utah, is in the Museum of Comparative Zoology, Cambridge, Massachusetts.

Eubaphe pumilata sp. nov.

(Plate fig. 54)

♀ 17-24 mm. Antennae, except basal segment, fore and mid-tibiae and tarsi fuscous; the remainder of the insect is uniformly orange buff.

Genitalia.—Ostium bursae with two serrate-edged, tapered processes projecting posteriorly, each two-thirds as long as the bursa copulatrix, which is ovate with the spining as shown in the figure.

Holotype and 5 Paratypes, all ♀ and in the British Museum: Cuba, Holguin, (*H.S. Parish*).

Eubaphe unicolor (Robinson)

(Text-figs. 20, 22; Plate fig. 52)

Euphanessa unicolor Robinson, 1869, Ann. N. Yk. Lyceum nat. Hist., 9:153, pl. 1:2.

Eudule hyalina Hulst, 1898, Canad. Ent., 30:114.

♂♀ 22-28 mm. Larger in size than the preceding species but identical in coloration.

Genitalia. Male.—Uncus broad and truncate. Valve almost rectangular, three times as long as broad; a slender, tapered and apically incurved process arises from one-third of the costal margin and extends almost to the apex of the valve. Juxta one-and-one-half times as broad as the valve, slightly longer than broad. Aedeagus one-and-one-half times as long as the distal margin of the valve, with two small, scobinate patches, one dorsad and the other ventrad of the apex. The vesica bears two cornuti, each a stout, tapered spine, one two-thirds and one one-half as long as the aedeagus; basad of the cornuti is a small sclerotized patch. The terminal sterna are as shown in the figure.

Female.—Ostium bursae with two smooth, tapered processes projecting posteriorly, each equal in length to two-thirds of the width of the bursa copulatrix, the posterior third of which is globular, sclerotized and spined half way round the circumference; the anterior two-thirds of the bursa copulatrix is a membranous sack with a sclerotized fold in the right side; the spining is as shown in the figure.

The type of *hyalina* is in the collection of Rutgers University, New Brunswick, New Jersey; that of *unicolor* has not yet been located.

Distribution.—Southern states of the United States of America; Mexico.

Eubaphe unicolor venustata subsp. nov.

♂♀ 30 mm. Larger than the nominotypical race and deeper in color, capucine orange replacing the orange buff. The depth and extent of the sclerotized fold in the bursa copulatrix in the female genitalia is one-half that of the nominotypical race; in other respects the genitalia of both sexes appear to be identical.

Holotype ♂ and Allotype ♀, both in the British Museum: Guatemala, Calderas, (*Champion*).

Eubaphe conformis (Walker)

(Text-figs. 19, 24; Plate fig. 53)

Ameria conformis Walker, 1854, List Lep. Ins. B. M., 2:555.

Leptidule sulcifera Warren, 1906, Proc. U. S. Nat. Mus., 30:467. Syn. nov.

Leptidule dulcifera Forbes, 1917, J. N. Yk. ent. Soc., 25:57.

♂ 25-26 mm.; ♀ 26-30 mm. Antennae, except basal segment, and legs, except coxae, fuscous.

Male.—On the underside of the forewing, in the anterior distal quarter of the cell, is a fold, the inner surfaces covered with a dense pad of tawny scales. Remainder of insect orange buff. The upperside of the hindwing has several long hairs arising from the base of the wing and extending half way to the discocellulars. *Female.*—On the forewing there is a narrow fuscous marginal band commencing just distad of mid-costa and extending round the apex to the tornus; the remainder of the insect is capucine orange.

Genitalia. Male.—The apex of the uncus is lunulate and broad; the socii are slender and equal in length to the width of the aedeagus. The valve is narrowed from both margins to form a waist at one-third; two processes arise from the costa; one from the base, which is short, stout and tipped with a cluster of spines and the second, which is slender, incurved and

tapered and arises just apicad of the first. The aedeagus has two scobinate patches, one ventrad and one dorsad of the apex. The vesica bears three cornuti, each a tapered spine; one is broad and stout and one-half as long as the aedeagus, the two others are more slender, one one-quarter and one one-fifth as long as the aedeagus; there is also a small sclerotized patch basad of the cornuti. The terminal sterna are as shown in the figure.

Female.—The ostium bursae bears two sclerotized processes, serrate-edged, tapered posteriorly and rounded anteriorly; two further processes, slender and tapered, project posteriorly, each one-half as long as the bursa copulatrix, which is almost rectangular, having the anterior fifth membranous and the remainder sclerotized. The spining is as shown in the figure.

The type of *conformis* is in the British Museum; that of *sulcifera* is in the United States National Museum.

Distribution.—Mexico, Jalapa, Orizaba and Vera Cruz; Guatemala, San Geronimo.

SECTION C

Eubaphe deceptata sp. nov.

(Text-figs. 25, 31; Plate fig. 55)

♂♀ 24-37 mm. Antennae, except basal segment, and legs, except coxae, fuscous; remainder of insect orange buff to capucine orange. In a few specimens the apex of the forewing is narrowly edged with fuscous.

Genitalia. Male.—Uncus arched with a minute pointed apex; socii equal in length to the width of the aedeagus, slightly broadened apically. Valve arc-shaped with a v-shaped incision midway along the distal margin extending for one-third of the width. A slender, tapered and incurved process arises from the costa at one-half. Aedeagus with a scobinate patch dorsad of the apex. There are three or four cornuti, each a stout sclerotized spine; one is one-half and the remainder one-third as long as the aedeagus; in addition there is a small sclerotized patch on the ventral side near the base. The terminal sterna are as shown in the figure.

Female.—Ventral surface of the ductus bursae as broad as long, shallowly bilobed posteriorly and broadly rounded anteriorly, strongly sclerotized. Bursa copulatrix twice as long as broad, membranous anteriorly, the remainder sclerotized; the spining is as shown in the figure.

Holotype ♂ and Allotype ♀: Mexico, Orizaba, xii. 1887, (*H. Salvin & F. D. Godman*).

Paratypes: 1 ♂, 1 ♀, Mexico; 2 ♂, 13 ♀, type locality; 3 ♀, Jalapa; 1 ♀, Ventanas, 2,000 ft.; 1 ♀. Atoyac; 1 ♀, Colima; 1 ♂, Guaymas; 1 ♀, Durango City; 2 ♀, Temax; 1 ♀, Coatepec; 1 ♀, Presidio; 1 ♂, Guatemala; 2 ♂, 3 ♀, San Geronimo; 1 ♂, 1 ♀, Barberena; 1 ♂, Honduras, La Cambre; 1 ♂, Nicaragua, Jinotega, 1,400 ft.; 1 ♂, Costa Rica, San Jose; 2 ♂, 1 ♀, Venezuela; 3 ♂, 2 ♀, Caracas; 2 ♀, Merida; 3 ♀, Peru; 4 ♀, Chanchamayo; 5 ♀, La Merced; 3 ♀, Chachapoyas; 1 ♀, Tabaconas; 1 ♂, 1 ♀, Pozuzu; 1 ♀, Cuzco; 4 ♀, Rio Colorado; 2 ♀, Bolivia, Chiquitos, S. Julian; 2 ♂, 1 ♀, Cochabamba; 2 ♀, La Paz; 2 ♀, Yungas de Corvico. All specimens are in the British Museum.

Eubaphe fieldi sp. nov.

(Text-figs. 27, 32; Plate fig. 57)

Superficially indistinguishable from the preceding species, *deceptata.*

Genitalia. Male.—The uncus is arched with the apex narrowly rounded; the socii are stout, twice as long as broad and separated by a distance equal to their length. Valve similar to *deceptata.* Manica very strongly spined, distinguishing this species at a glance from its allies. The aedeagus has two scobinate patches, one ventrad and one dorsad of the apex. The vesica has scobinate ridges extending lengthwise at the center and one cornutus, one-third as long as the aedeagus; in addition there is a slender sclerotized patch midway along one side.

Female.—Posterior edge of the ductus bursae twice as broad as the anterior one. The arrangement of the spining in the bursa copulatrix, as will be seen from the figure, is quite distinctive.

Holotype ♂ and Allotype ♀: Venezuela, San Esteban, vi. 1909, *(S.M.Klages).*

Paratypes: 2 ♀, Venezuela; 2 ♂, 2 ♀, type locality; 2 ♂, Ciud. Bolivar; 1 ♂, 6 ♀, Colombia, Santa Maria. All specimens are in the British Museum.

Eubaphe cupraria (Walker)

(Text-figs. 4, 26, 33; Plate fig. 56)

Crocota cupraria Walker, 1854, List Lep. Ins. B. M., 2:536.

Eudule cupraria, Prout, 1910, Trans. ent. Soc. Lond., 43:231.

♂♀ 23-28 mm. Identical in coloration with *deceptata.* Previously thought to be widely distributed in Central and South America, but appears to be confined to the northern provinces of Brazil. Prout in his paper, "The Geometridae of the Argentine Republic," in 1910 states that "the specimen which bears Walker's type label was from Peru"; this specimen must certainly have been erroneously labelled, for Peru is not mentioned in the localities listed by Walker; further, the specimen bearing the type label is one of the two specimens from Brazil presented by E. Doubleday, a male. This is hereby designated

lectotype. Of the remaining specimens, which together with the lectotype made up Walker's original series, only one other specimen remains in the British Museum. It is a male, one of the specimens presented by W. F. Evans, the locality of which is unknown; it is in fact a different species from the type, being *E. hesperina* Burmeister, and probably came from S. Brazil.

Genitalia. Male.—Apex of uncus narrowly rounded; socii stout, a little longer than broad. Valve arc-shaped with a V-shaped incision at two-thirds of the distal margin, extending for one-third of the width. A slender, tapered and incurved process arises from one-half of the costa. The aedeagus has a strongly scobinate patch dorsad of the apex; the vesica bears one cornutus two-thirds as long as the aedeagus and in addition there is a small sclerotized patch about halfway along one side. The seventh sternum is similar to that of *deceptata;* the eighth differs in being broader based and having the apices sharply incurved.

Female.—Ductus bursae sclerotized, almost square and smooth. Bursa copulatrix twice as long as broad, almost rectangular with a slight membranous projection posteriorly at the right side; the spining is as shown in the figure.

Distribution.—Brazil, Amazons, Gran Pará, Maranhao, Pernambuco and Minas Geraes.

Eubaphe hesperina (Burmeister)

(Text-figs. 28, 36; Plate fig. 58)

Eudule hesperina Burmeister, 1878, Descr. Phys. Rep. Argentina, 5(1):428.

Eudule nanora Schaus, Proc. ent. Soc. Washington, 31:52, pl. 3:4. Syn. nov.

The fine blackening of the distal half of the costal margin of the forewing distinguishes many specimens of *hesperina* at a glance from the closely related *deceptata, fieldi* and *cupraria.* Unfortunately not all specimens of *hesperina* are so marked, but on the other hand no specimen of the other three species has yet been noted with a blackened costa; in other respects *hesperina* is indistinguishable from them.

Burmeister described the species from several examples from Buenos Aires and Cordoba; of the four original specimens in the collection of the Argentine Museum of Natural History in Buenos Aires, I designate as lectotype the specimen labelled "Buenos Aires, Pergamino." The specimens from Cordoba are *Eudule trichoptera* Perty.

Genitalia. Male.—*E. hesperina* differs principally from its closest relatives in the aedeagus. The vesica bears two cornuti; one is tapered and strongly sclerotized and one-half as long as the aedeagus; the second, one-quarter as long as the aedeagus, is coarsely scobinate. The terminal sterna are as shown in the figure.

Female.—Ductus bursae strongly sclerotized, broadened posteriorly. Bursa copulatrix three times as long as broad with narrow ridges of spining at the right side; at the right side posteriorly is a heavily spined and sclerotized projection, larger than and overlapping the ductus bursae.

The type of *hesperina* is in the Argentine Museum of Natural History; that of *nanora* is in the United States National Museum.

Distribution.—Brazil, Sao Paulo, Parana and Sta. Catharina; Bolivia; Argentine; Paraguay.

Eubaphe integra (Walker)

(Text-figs. 29, 35; Plate fig. 59)

Ameria integra Walker, 1866, List Lep. Ins. B. M., 35:1893.

Leptidule antithesis Dyar, 1914, Proc. U. S. Nat. Mus., 47:228. Syn. nov.

♀ 30-34 mm. Antennae, except basal segment, fore- and mid-tibiae and tarsi fuscous; head, thorax and abdomen orange buff to capucine orange. The male has two dense patches of olive lake scaling on the otherwise orange buff wings; one is on the mid-underside of the forewing, bounded anteriorly by the upper median and posteriorly by vein *A*1. The second on the mid-upperside of the hindwing is bounded anteriorly by the costal vein and posteriorly by the lower median. Both wings of the female are uniformly orange buff to capucine orange. Both sexes of this species may be recognized by the neuration of the hindwing, in which the vein *Sc* and *M*1 arise separately from the cell; in all the other species so far known in the genus these veins are long-stalked.

Genitalia. Male.—Uncus broadly arched; socii rather longer than broad, close together, almost fusing basad. Distal margin of the valve shallowly incised at one-half. Aedeagus with a scobinate patch dorsad of the apex. Vesica lightly scobinate in middle with one slender, tapered cornutus, rather less than one-half as long as the aedeagus, and a small sclerotized patch midway along one side. The terminal sterna are as shown in the figure.

Female.—Ductus bursae twice as broad posteriorly as anteriorly, sclerotized and ribbed. Bursa copulatrix three times as long as broad, strongly sclerotized posteriorly, membranous anteriorly; the spining is as shown in the figure.

The type of *integra* is in the British Museum; that of *antithesis* is in the United States National Museum.

Distribution.—Honduras; Panama; Colombia; Venezuela; French and Dutch Guiana; Brazil, Amazons and Para.

Eubaphe orfilai sp. nov.

(Text-figs. 30, 34; Plate fig. 60)

♂ 22-23 mm.; ♀ 30 mm. Similar in coloration to the preceding species, except for the antennae which are concolorous with the wings.

Genitalia. Male.—Uncus narrowly arched; socii fused basad then outwardly curved, half as long as the aedeagus. Valve with a V-shaped incision at two-thirds of the distal margin, extending one-half of the way across its width. A curved, slender process arises from one-half of the costal margin and extends beyond the apex of the valve. Aedeagus slightly bowed with a scobinate patch dorsad of the apex. The vesica has two stout, tapered cornuti, each about one-half as long as the aedeagus. The seventh and eighth sterna are fused and shaped as shown in the figure.

Female.—Ductus bursae rather broader than long, sclerotized and ribbed. Bursa copulatrix pyriform; the anterior quarter is membranous, the remainder strongly sclerotized and spined.

Holotype ♂: Brazil, Matto Grosso, 1886, (*P. Germain*).

Allotype ♀: Brazil, Parana, Ignassa, x-xii. 1922.

Paratypes: 1 ♂, Brazil; 1 ♂, Matto Grosso; 1 ♀, Sao Paulo, Anhanggahay; 2 ♂, C. Paraguay; 4 ♀, Sapucay. All specimens are in the British Museum.

Eubaphe weyenberghii (Snellen)

(Text-figs. 37, 39; Plate fig. 62)

Eudule weyenberghii Snellen, 1878, Bol. Acad. Nat. Cien. Cordoba, 2(4):390.

Eudule sombreata Dognin, 1893, Ann. Soc. ent. Belg., 37:424. Syn. nov.

Eudule weyenberghii, Prout, 1910, Trans. ent. Soc. Lond., 43:232.

The antennae, except for the basal segment, the tibiae and the tarsi are fuscous. In the female the remainder of the insect is capucine orange. The male is conspicuous in having both surfaces of both wings shaded with fuscous; the shading extends over the whole of the forewing, almost to the distal margin; on the hindwing the shading is more intense and extends across the whole width of the wing from the costa to vein *M*2.

Genitalia. Male.—Uncus narrowly arched; socii wanting. Valve incised for one-third of its width at one-half of the distal margin, which is straight-edged in the anterior half and curved in the posterior half. Aedeagus with a strongly scobinate patch dorsad of the apex. Vesica scobinate in longitudinal ridges with two cornuti, one one-half and one two-thirds as long as the aedeagus; in addition there is a small sclerotized patch midway along one side. The terminal sterna are as shown in the figure.

Female.—Ductus bursae almost twice as long as broad, strongly sclerotized and smooth. Bursa copulatrix long and slender, five times as long as broad; the posterior half is strongly sclerotized with a cluster of spines at the right side posteriorly; the anterior half is lightly sclerotized, ribbed and thickly spined.

The type of *weyenberghii* is in the Leiden Museum; that of *sombreata* is in the United States National Museum.

Distribution.—Argentine; Paraguay; Brazil; Bolivia; Peru; Ecuador.

Eubaphe medea (Druce)

(Text-figs. 38, 40; Plate fig. 63)

Leptidule medea Druce, 1885, Biol. Cent. Amer., Zool., Lep. Het., 1:139, pl. 13:12.

Leptidule medea Druce, 1897, Biol. Cent. Amer., Zool., Lep. Het., 2:403.

In the male the wings are slightly hyaline, a pale maize yellow with a patch of dense scaling the color of raw sienna in the middle of the forewing, bounded anteriorly by the upper median and posteriorly by vein *A*2. There is also a narrow fold at mid-costa enclosing similarly colored dense scales. The female is identical in coloration with the preceding species but recognizable by the neuration of the forewing, in which vein *Sc*2 arises from three-quarters of the upper margin of the areole, midway between the point of origin of *Sc*1 and the stalk of *Sc*3-5.

Genitalia. Male.—Uncus broadly rounded; socii short, stout and almost square, separated by a distance equal to their width. Valve incised for one-third of its width just beyond one-half of the distal margin. Aedeagus with a strong scobinate patch dorsad of the apex. Vesica with a cluster of one long and five short spines apicad and a dense cluster of small spines at the middle; in addition there is a small sclerotized patch at about half-way along one side. The terminal sterna, which differ from those of *weyenberghii* only in their larger size, are figured.

Female.—Ductus bursae strongly sclerotized, slightly tapered anteriorly, two-and-a-half times as long as broad; there is a U-shaped depression in the posterior edge. Bursa copulatrix slender, as long as the ductus bursae, tapered anteriorly and strongly sclerotized; the spining is as shown in the figure.

The type is in the British Museum.

Distribution.—Guatemala; Honduras; Costa Rica; Panama.

Eubaphe aeetes (Schaus)

(Text-fig. 41)

Leptidule aeetes Schaus, 1889, Ent. Amer., 5:191.

Leptidule aeetes Druce, 1897, Biol. Cent. Amer., Zool., Lep. Het., 2:403.

"Primaries ochreous, darkest on the margins and with a darker band crossing the wing from the costal margin near the apex to the middle of the inner margin. Secondaries ochreous, slightly hyaline, darkest around the outer margins. Head, thorax and abdomen ochreous. Antennae black. Expanse 21 mm."

Closely related to the preceding species but not synonymous with it as suggested by Druce. Mr. Field, who has kindly examined the genitalia of the unique male type, tells me that the principal difference between the two species is to be found in the aedeagus; the dense cluster of spines at the middle in *medea* is completely wanting in *aeetes;* the cluster of six spines at the apex in *medea* is represented by a cluster of about twenty-five in *aeetes*. The figure of the seventh and eighth sterna is from an outline supplied by Mr. Field.

The type is in the United States National Museum.

Distribution.—Mexico, Vera Cruz.

Eubaphe lobula Hübner

(Text-figs. 42, 43; Plate fig. 61)

Eubaphe lobula Hübner, 1823, Zuträge z. exot. Schmett., 2:20, pl. 52:299, 300.

Eudule lobiformis Druce, 1899, Ann. Mag. nat. Hist., (7) 3:294.

Eudule lobiformis Prout, 1910, Trans. ent. Soc. Lond., 43:232.

The males are at once recognizable by the fold on the forewing commencing at one-half and extending to three-quarters of the costa. The females, too, may be distinguished by the position of vein *Sc*1 in the forewing, which arises close to the distal angle of the areole near to the stalk of veins *Sc*2-5. The antennae, except for the basal segment, are fuscous; the remainder of the insect is uniformly capucine orange.

Genitalia. Male.—Valve with a V-shaped incision in the distal margin, extending for one-half of the width. The socii are stout and as broad as long. The aedeagus has a scobinate patch dorsad of the apex. The vesica is sclerotized in the posterior half with one cornutus, similar in shape to that of *cupraria,* but slightly less than one-half as long as the aedeagus; in addition there is a small sclerotized patch midway along one side. The terminal sterna are as shown in the figure.

Female.—Ductus bursae twice as broad as long, sclerotized and ribbed. Bursa copulatrix ovate, twice as long as broad, sclerotized and with spining as shown in the figure.

The type of *lobula* is not in the Hübner collection in the Hofmuseum in Vienna and it may prove to be lost; that of *lobiformis* is in the British Museum.

Distribution.—Costa Rica; Panama; Colombia; Venezuela; Brazil as far south as Sao Paulo; Paraguay.

Eubaphe tritonia (Druce)

Eudule tritonia Druce, 1885, Proc. zool. Soc. Lond., 1885:524, pl. 32:9.

Three specimens of the original series of four females of this species are without abdomens and the genitalia dissected from the fourth proved to be imperfect. The ductus bursae is well sclerotized, twice as long as broad, slightly narrowed anteriorly and truncate posteriorly; the bursa copulatrix is membranous with a narrow band of spines close to the neck. The sack is unfortunately shrivelled, but the genitalia appear to show relationship with the *rhotana-tripunctata* section of the genus.

The type is in the British Museum.

Distribution.—Ecuador.

The following species, similar in appearance to *cupraria* Walker and usually associated with that species, has been transferred to the genus *Eudule* on the basis of the structure of the genitalia:

Eudule trichoptera (Perty)

Lithosia trichoptera Perty, 1833, *in* Spix, Delectus. Anim. arcticul. Brazil, (3):161, pl. 32:5.

Eudule costata Warren, 1897, Novit. zool., 4:456. Syn. nov.

Eudule aurata Schaus (1892, Proc. Zool. Soc. Lond., 1892:284) has been found to be synonymous with *Oncopus citrosa* Geyer (1832, Zuträge z. exot. Schmett., 4:18, pl. 114:661, 662) and has been transferred to the subfamily Sterrhinae.

EXPLANATION OF THE PLATES

Plate I

Fig. 44. *E. eulathes,* Female genitalia.
Fig. 45. *E. lineata,* Female genitalia.
Fig. 46. *E. rhotana,* Female genitalia.
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Fig. 52. *E. unicolor,* Female genitalia.
Fig. 53. *E. conformis,* Female genitalia.
Fig. 54. *E. pumilata,* Female genitalia.

Plate II

Fig. 55. *E. deceptata,* Female genitalia.
Fig. 56. *E. cupraria,* Female genitalia.
Fig. 57. *E. fieldi,* Female genitalia.
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Fig. 59. *E. integra,* Female genitalia.
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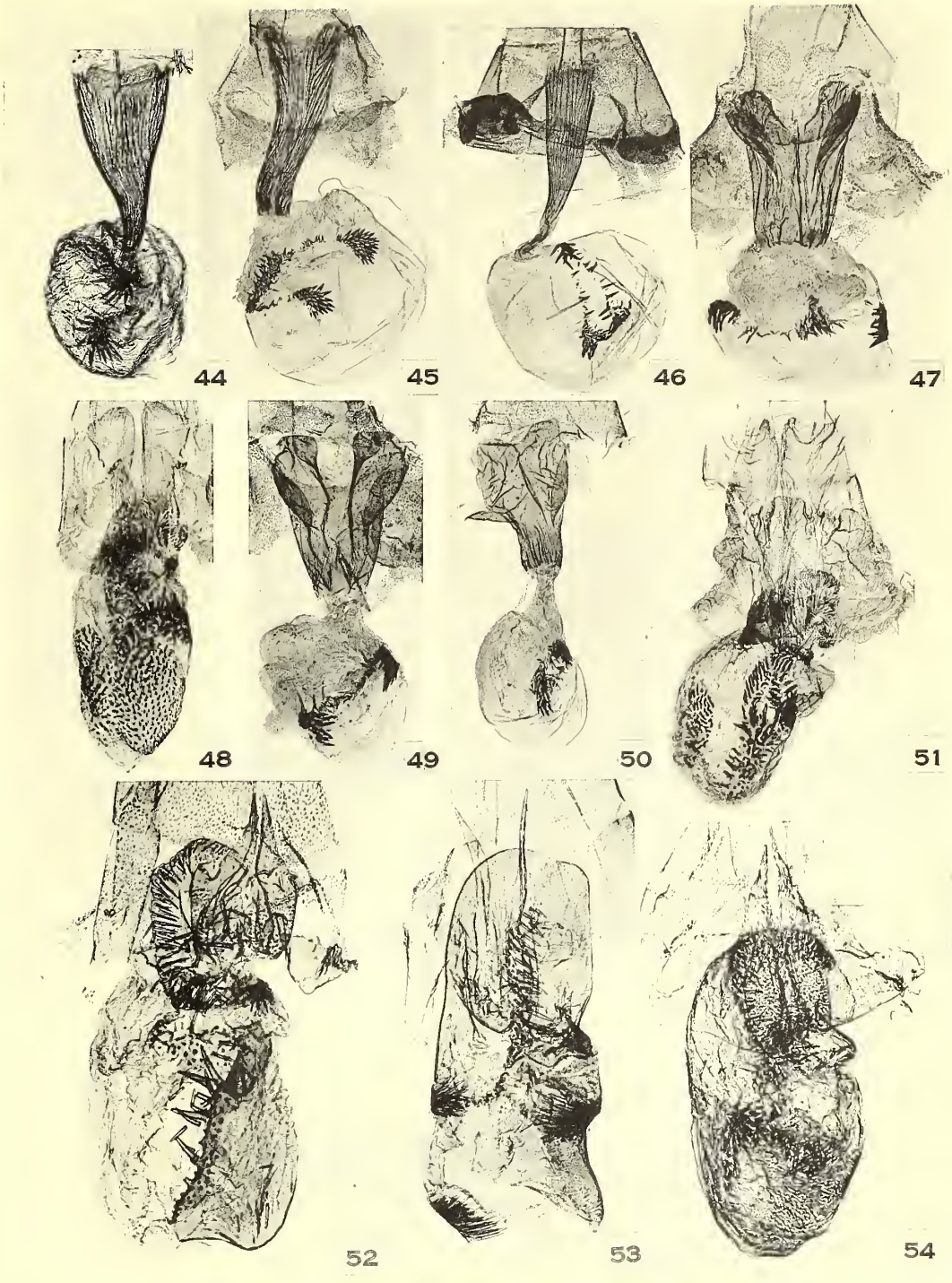
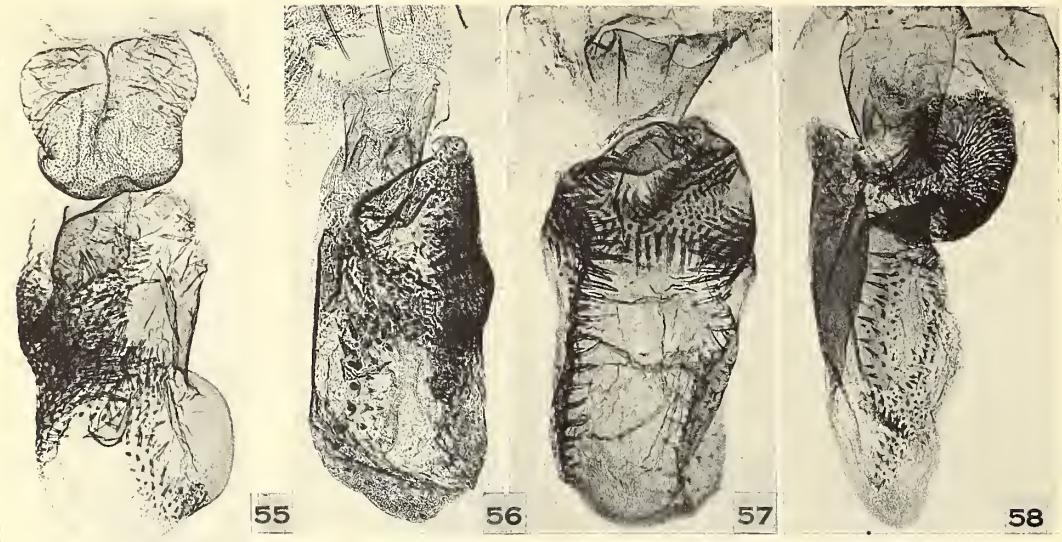


A REVISION OF THE GENUS EUBAPHE (LEPIDOPTERA: GEOMETRIDAE)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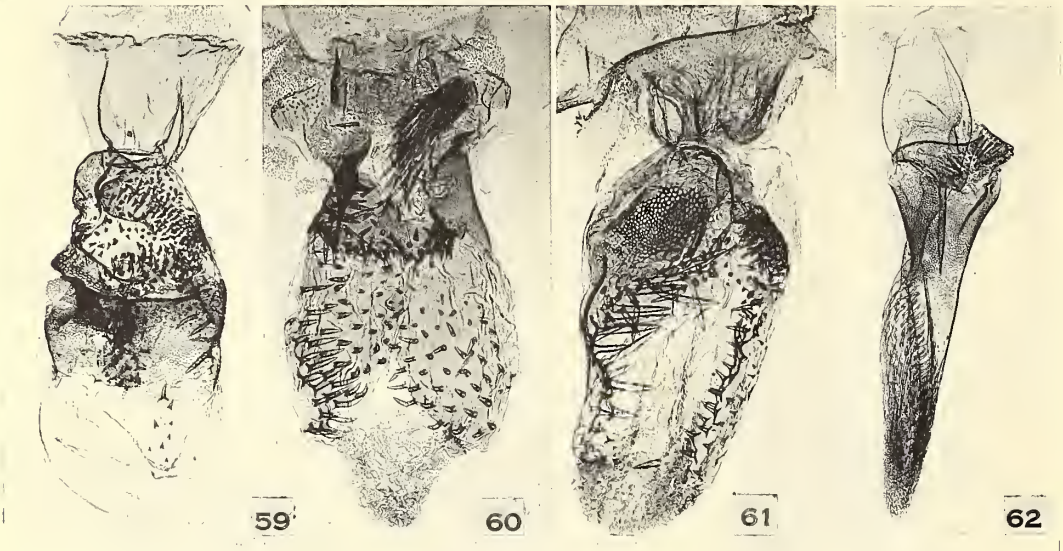
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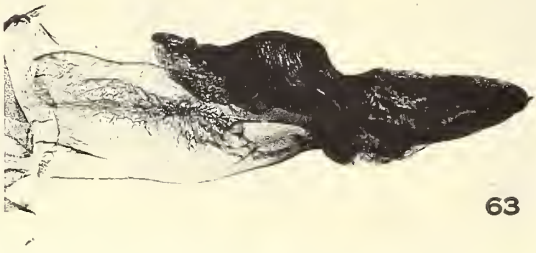
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